EFFECTS OF RESISTANCE IN *PHASEOLUS VULGARIS* ON DEVELOPMENT OF *MELOIDOGYNE* SPECIES

By

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Abstract of Dissertation Presented to the Graduate School
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EFFECTS OF RESISTANCE IN *PHASEOLUS VULGARIS* ON DEVELOPMENT OF *MELOIDOGYNE* SPECIES

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Root-knot nematodes, *Meloidogyne* spp., are major limiting factors in the production of common bean, *Phaseolus vulgaris*. Use of resistant *P. vulgaris* germplasm has potential for management of root-knot nematodes. Mechanisms of resistance of two genetic resistance systems and effects of temperature on resistance expression were studied using a susceptible cultivar 'Black Valentine' and either resistant cultivar 'Nemasnap' (gene system 1) with *M. incognita* race 2, or germplasm G1805 (gene system 2) with *M. arenaria* race 1. Mechanisms of resistance and effects of pre- and post-inoculation temperatures (24°C and 28°C) were evaluated at specific harvest dates by determining nematode populations within roots and the proportion of each population within distinct developmental stages.

Since similar numbers of juveniles penetrated roots of resistant plants compared with susceptible Black Valentine, pre-penetration mechanisms of resistance were not expressed in either Nemasnap or G1805. Resistance was expressed by post-penetration

mechanisms, which resulted in smaller populations of nematodes and their delayed development in resistant plants compared with susceptible plants.

Resistance was effective at all temperature regimes examined, with smaller populations of nematodes in roots of Nemasnap and G1805 compared to Black Valentine. Pre-inoculation temperature, *per se*, did not modify resistance expression in Nemasnap or G1805 to later infections by *M. incognita* or *M. arenaria*, respectively. However, post-inoculation temperatures had pronounced effects on the development of *Meloidogyne* spp. in all genotypes. The more rapid development of nematodes to adult stages at the higher temperature of 28°C compared with 24°C, in both the resistant and the susceptible germplasms, indicates that the effect of post-inoculation temperature on nematode development is direct, rather than via resistance expression of either gene system 1 or gene system 2. Results of studies at higher temperatures demonstrated that resistance in Nemasnap and in G1805 also was stable at 30°C and 32°C.

Tests with Florida populations of root-knot nematodes identified potentially virulent pathotypes of *M. incognita* race 3 and *M. javanica* in their resistant *P. vulgaris* hosts containing gene systems 1 and 2, respectively. Thus it is critical to assess the performance of resistant germplasm to a wide range of geographically localized populations of nematode species.

CHAPTER 1
REVIEW OF LITERATURE

The common bean, *Phaseolus vulgaris* L., has long been used as a food crop. It was domesticated in South America, arrived in Africa with the slave trade, and reached Europe by the sixteenth century (Hall, 1991). Common bean plants with parchmented pods are harvested when mature for dry beans. Plants with fleshy pods are harvested immature as fresh green or snap pods. *Phaseolus vulgaris* is a warm season, annual plant, grown widely throughout subtropical and temperate regions; since high temperatures and rainfall promote disease and flower abortion, it is grown in tropical areas during the cool, dry seasons. The common bean is susceptible to a wide range of foliar and root diseases, caused by fungi, bacteria, viruses, and nematodes

Some of the most important nematode parasites of beans are species of *Meloidogyne*, the root-knot nematodes, which are associated with roots of beans throughout the world. *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood are the most prevalent species of root-knot nematodes in subtropical and tropical regions. Root-knot nematodes have been reported to cause yield losses in beans as high as 50-90% (Abawi and Varon de Aguldo, 1989; Mullin *et al.*, 1991b, Ngundo and Taylor, 1974). Only the infective, second-stage juveniles of these obligate endoparasites are free in the soil. These juveniles penetrate roots behind the root tip and migrate intercellularly and intracellularly to the stele, where they become sedentary with their heads inserted into the vascular system. Nematode secretions stimulate plant cell hypertrophy and hyperplasia. Specialized giant cells, which form near the head of the juvenile in response to feeding, lead to the formation of root swellings, galls or knots. As giant cells form, juveniles enlarge and molt to adults. Vermiform males migrate out of the root whereas pyriform females protrude through the root surface. Above-ground symptoms of root-knot nematode damage on beans are nonspecific chlorosis, stunting, and wilting. Galls or knots on the roots are diagnostic symptoms. It has been reported that infection by root-knot nematodes suppresses root growth, which reduces the size of the root system, and restricts water and nutrient uptake and balance (Hussey, 1985).

The high cost and restricted use of nematicides and inconsistency of cultural control methods for management of root-knot nematodes in beans have led to screening studies for the identification and development of resistant bean germplasm. Use of resistance in beans may provide an economic and environmentally safe management tool for root-knot nematodes, especially in developing countries where availability of other control methods is limited.

Resistance to root-knot nematode in common beans was first reported by Isbell (1931), who collected pole bean varieties from farm gardens and tested them for resistance to root-knot nematodes, known at that time as *Heterodera marioni* (Cornu) Goodey. His work resulted in the release of two selections with resistance to root-knot nematodes, Alabama Selection #1 and Alabama Selection #2. Barrons (1939) followed up the work of Isbell by looking at the mechanism of resistance and found that juveniles equally penetrated roots of resistant Alabama #1 and the susceptible control, Kentucky Wonder. However, juveniles and galls failed to develop in Alabama #1. In a second paper, he described the inheritance of root-knot nematode resistance in bean (Barrons, 1940). By hybridizing Alabama #1 with adapted varieties he was able to follow the resistant trait in the progeny generations. His F_2 generation displayed a segregation ratio of 11 susceptible:4 intermediate:1 resistant. From these results he concluded that root-knot nematode resistance in Alabama #1 was inherited as a double recessive trait, and that this trait was quantitatively controlled. Two dominant genes present in a plant result in susceptibility, whereas one dominant gene gives rise to a phenotype with intermediate resistance.

In 1964, after Chitwood reclassified root-knot nematodes into *Meloidogyne* spp., Blazey *et al.* (1964) conducted a study with 55 *P. vulgaris* varieties to determine to which specific species of *Meloidogyne* the beans were resistant, and how this resistance was inherited. They identified seven bean varieties, including Alabama #1, with resistance restricted to *M. incognita*, but susceptible to *M. hapla* Chitwood, *M. javanica, M. arenaria* (Neal) Chitwood, and *M. arenaria thamesi* Chitwood. The results of their study on the inheritance of resistance were in agreement with those of Barrons (1940), in that resistance was controlled by the interaction of two independent recessive genes. Also, they found that the resistance in the two varieties used as the resistant parents, Wingard Wonder and Springwater Half Runner, was controlled by the same gene system as Alabama #1. By following progeny to the F_3 and F_4 generations, Hartmann (1971) concluded that the inheritance of *M. incognita* resistance from Alabama #1 in variety Manoa Wonder was better explained by at least a three-gene system, with three or more pairs of genes acting equally. Interaction of these genes controls resistance, with a certain number of genes for susceptibility being necessary before all resistance is lost.

More recently, two new sources of resistance to *M. incognita* have been identified in the plant introduction germplasm, PI 165426 and PI 165435, as the result of an extensive germplasm survey by Fassuliotis *et al.* (1970). Omwega *et al.* (1989) reported that the resistance identified in these plant introductions was similar to that in Alabama #1. The gene system in these lines conferred resistance to *M. incognita* races 2, 3, and 4. Omwega *et al.* (1989) also identified a second gene system conferring resistance to *M. incognita* race 1, *M. javanica*, and *M. arenaria* races 1 and 2. This resistance, identified in two accessions from Mexico, G1805 and G2618, and lines A252, A315, A328, A443, and A445 bred from them, was shown to be controlled by a single dominant gene (Omwega *et al.*, 1990b). Currently, researchers from the University of California, at Davis and Riverside, are working to incorporate and combine these two gene systems in beans (P.A. Roberts, personal communication).

Resistance in plants generally can be described as the effect of host genes to prevent or restrict nematode reproduction. Tolerance refers to the ability of the plant to withstand nematode injury and is independent of resistance (Trudgill, 1991). Mechanisms of resistance can be divided into pre- and post-infectional mechanisms (J. S. Huang, 1985). Pre-infectional mechanisms of resistance include the formation of root exudates, which can be differentially repellent to nematodes, and the formation of nematicidal compounds. Post-infectional resistance also may be effected by chemical compounds, pre-formed or formed in response to nematode attack, sometimes resulting in a hypersensitive reaction. Several phenolic compounds and their precursors have been reported to be involved in resistance reactions (Brueske, 1980). Phytoalexins, formed in plants in response to microbial invasion, also have been reported to be involved in resistant reactions (Kaplan *et al.*, 1980). Inability of the host to provide certain necessary nutrients may play a role in post-infectional resistance (J. S. Huang, 1985; Giebel, 1974).

Some early work has looked at the mechanisms of resistance in common bean. Barrons (1939) reported that penetration by root-knot nematode juveniles was equal in both the susceptible and resistant cultivars, but that juvenile development and gall formation were absent in the resistant variety Alabama #1. Fassuliotis *et al.* (1970) studied the penetration of *M. incognita* and the histological response in susceptible and resistant bean roots. They also found no differences in the penetration of juveniles into Black Valentine (susceptible control) and PI 165426 or breeding line B3864 (resistant lines). In the roots of susceptible Black Valentine, the juveniles stimulated formation of giant cells as early as four days after inoculation, which resulted in extremely large giant cells and noticeable galls by nine days after inoculation. After four days, only a few, small giant cells formed in the infected B3864 roots, with little hyperplasia and hypertrophy. Most of these giant cells were necrotic within nine days after inoculation and only slight root swellings were observed. It was concluded that resistance to *M. incognita* from PI 165426 was related to a hypersensitive reaction. From this early work, it can be concluded that post-infectional rather than pre-infectional mechanisms of resistance are involved in this host-parasite interaction.

In addition to the effects of resistance genes in beans on root-knot nematode populations, the effects of nematode infection on beans also have been studied. Melakeberhan *et al.* (1985-1988) studied the effects of *M. incognita* infection on plant tissue nutrient concentration, physiology, growth and yield of beans. In young bean plants photosynthetic rates and photosynthesis to respiration ratios decreased with increasing inoculum levels of nematodes. Nematode infection increased concentrations of calcium, copper, and iron in the shoot and potassium in the root, whereas concentrations of copper and zinc decreased in the roots. However, total nutrient concentration per plant decreased due to nematode infection (Melakeberhan *et al.*, 1987, 1985b). In another study it was shown that chlorophyll content, plant dry weight and numbers of flowers, pods and seeds also were lower in infected plants than in non-inoculated plants (Melakeberhan *et al.*, 1986, 1985a). However, the addition of potassium nitrate decreased yield reduction caused by nematodes in beans (Melakeberhan *et al.*, 1988). Wilcox and Loria (1986), studying the effects of *M. hapla* on water relations, growth, and yield of two snap bean cultivars, found that leaf area, xylem water potential, and yield were reduced by nematode infection, and that the two cultivars differed in their response to *M. hapla*. They concluded that nematode tolerance in bean cultivars is related to the effect of nematodes on water uptake. In these studies it was shown that root-knot nematode infection of susceptible bean plants does affect the physiology of the plants as related to growth and yield.

Susceptible plants thus provide nematodes with the energy required to sustain growth and reproduction at a cost to the plant. However, mechanisms conferring resistance in plants may act in a way to prevent nematodes from obtaining all the energy required for normal development (Powers and McSorley, 1993). Feeding of root-knot nematodes has been shown to be more expensive in energy costs to susceptible plants than to resistant plants (Melakeberhan and Ferris, 1989). However, resistance mechanisms may themselves by energy requiring processes and may result in the re-allocation of energy from vegetative and reproductive growth to the site of nematode infection in the roots.

Temperature plays an important role in the expression of plant resistance to root-knot nematodes. In general, resistance to nematodes decreases as temperature increases (Carter, 1982; Dropkin, 1969). An early report on the effect of temperature on root-knot nematode development in beans determined that a minimum number of heat units are necessary for nematode development, and that development above 12°C is directly proportional to accumulated heat units (Townsend, 1937). Vicente *et al.* (1987), studying the influence of temperature and soil type on the histopathology of *M. incognita* in susceptible beans, also found that numbers of the more mature stages were greater at higher temperatures. Ngundo and Taylor (1975) reported that higher temperature increased the penetration of juveniles of *M. incognita* and *M. javanica* into bean roots.

Fassuliotis *et al.* (1970) studied the effect of temperature on the expression of resistance to *M. incognita* race 3 in a susceptible cultivar, resistant PI 165426, and two selections of the resistant breeding line B3864. All roots showed light galling at the lower temperature (16°C); more galls were visible as temperatures increased to 21°C and 28°C. Production of females and egg masses also increased with the increase in temperature. However, the numbers of females that developed in the roots of the resistant lines at the higher temperatures were still less than those in the susceptible cultivar. Omwega *et al.* (1990a) looked at the effect of temperature on the expression of resistance of the two gene systems conferring resistance to the various *Meloidogyne* species and races. They found that resistance to *M. incognita* race 1 and *M. javanica*, in germplasm with gene system 2, was stable at temperatures of 24° C to 30°C; however, *M. javanica* populations increased as temperatures increased from 26°C to 30°C. The germplasm with gene system 1 were heat stressed at 27°C, and resistance to *M. incognita* race 2 was lost at 30°C in Alabama #1 and PI 165435, but remained somewhat effective in PI 165426.

Mullin *et al.* (1991a), working with bean line A211 resistant to *M. incognita*, examined the effect of length of exposure of plants to high or low temperatures on resistance expression with respect to nematode reproduction and root galling. They reported that the minimum exposure times required to cause significant increases in nematode reproduction and root galling, after inoculation at 30°C and before incubation at 22°C, were 3 and 16 days, respectively. The minimum exposure times necessary to continue the resistant reaction, after inoculation at the low temperature before high temperature treatment, were 16 and 23 days for nematode reproduction and root galling, respectively. They also found that resistance to nematode reproduction was affected at a lower temperature, 24°C, than was resistance to root galling, 26°C. These results indicate that temperature modification of resistance expression is important, especially during the infection process.

Based on published reports in the literature, there is a large body of knowledge on resistance to root-knot nematode in beans, the effects of nematodes on bean growth and development, and the effects of temperature on resistance expression. However, little is known about the mechanisms of the gene systems conferring resistance in beans. The overall focus of this research is to understand the relationship between *Meloidogyne* spp. and the bean plants in which resistance is expressed. These studies should provide information useful to understanding resistance at a more fundamental level. The specific objectives of this study are to evaluate the effects of the two resistance gene systems on nematode infection and development, to determine the effects on bean growth of resistant accessions infected with root-knot nematodes, and to assess the effect of temperature on resistance expression of the two gene systems.

CHAPTER 2

EFFECTS OF RESISTANCE IN GERMPLASM OF *PHASEOLUS VULGARIS* ON PENETRATION AND DEVELOPMENT OF *MELOIDOGYNE INCOGNITA* RACE 2 AND *MELOIDOGYNE ARENARIA* RACE 1

Introduction

Common bean, *Phaseolus vulgaris* L., is a susceptible host of root-knot nematodes (*Meloidogyne* spp.), which have been reported to cause yield losses in bean as high as 50-90% (Abawi *et al*., 1989; Mullin *et al*., 1991b; Ngundo and Taylor, 1974). High cost and restricted use of nematicides, and inconsistency of cultural control methods for management of root-knot nematodes in beans have led to screening studies for the identification and development of resistant bean germplasm. Use of resistance in beans may provide an economic and environmentally safe management strategy for root-knot nematodes, especially in developing countries where availability of other control methods is limited.

Resistance to root-knot nematodes in *P. vulgaris*, identified during germplasm screening studies, recently has been reported to be controlled by one of two genetic systems (Omwega *et al*., 1989). Gene system 1, found in the cultivars Alabama #1 and Nemasnap, and in the genotype PI 165426, conditions resistance to *M. incognita* (Kofoid and White) Chitwood races 2, 3, and 4, and is controlled by the interaction of two or more independent recessive genes (Blazey *et al*., 1964; Fassuliotis *et al.*, 1970). Gene system 2, found in the Mexican accessions G2618 and G1805, confers resistance to *M. incognita* race 1, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood races 1 and 2. Resistance in gene system 2 is controlled by a single dominant gene (Omwega *et al*., 1990b). Resistant cultivars, developed before the specific nature of these gene systems was understood, may be limited in use because of poor results due to the presence of mixed populations of *Meloidogyne* spp. in some agricultural soils.

Resistance in plants generally can be described as the effect of host genes to prevent or restrict nematode reproduction, whereas tolerance is the ability of the plant to withstand nematode injury and is independent of resistance (Trudgill, 1991). Mechanisms of resistance can be divided into pre- and post-infectional mechanisms (J. S. Huang, 1985). Chemical compounds have been reported to be involved in both pre- and post-infectional resistance mechanisms (Breuske, 1980; Kaplan *et al.*, 1980). Inability of the host to provide certain necessary nutrients also may play a role in post-infectional resistance (Giebel, 1974; J. S. Huang, 1985). The focus of this research was to understand the relationship between *Meloidogyne* spp. and the bean germplasm in which resistance is expressed. These studies should provide information useful to understanding resistance at a more fundamental level.

Resistance mechanisms in *P. vulgaris* have been studied only in lines containing gene system 1. Barrons (1939) reported that penetration by root-knot nematode juveniles was equal in both a susceptible and resistant cultivar, but that nematode development and gall formation were absent in the resistant variety, Alabama #1. Fassuliotis *et al.* (1970) found no differences in the penetration of infective juveniles of *M. incognita* into the roots of susceptible and resistant plants. However, differences were observed in giant cell formation and galling responses in the roots of the susceptible and resistant lines.

Previous work did not evaluate the effects of resistance on development of root-knot nematodes in gene system 1 containing bean germplasm; and the resistance mechanisms in bean germplasm containing gene system 2 have not been studied at all. Thus, the objectives of this study were to evaluate the effects of the two genetic resistance systems on nematode infection and development, and to determine the effects of nematode infection on growth of resistant and susceptible bean genotypes.

Materials and Methods

All experiments were conducted at the University of Florida in Gainesville, Florida, during 1994-1995. The protocol for all experiments was similar.

The population of *M. incognita* race 2 used as inoculum in these studies was originally obtained from field-grown tobacco (*Nicotiana tabacum* L.); the population of *M. arenaria* race 1 used in these studies was originally isolated from field-grown peanut (*Arachis hypogea* L.). These two populations were increased in greenhouse cultures of tomato (*Lycopersicon esculentum* Mill. cv Rutgers) and peanut (cv Florunner), respectively. Nematode eggs were extracted from 2-month-old plants with NaOCl (Hussey and Barker, 1973). Hatched second-stage juveniles were collected from Baermann funnels over 72 hours to obtain populations of infective juveniles of similar age.

The *P. vulgaris* germplasms used in theses studies were Black Valentine, obtained from Phipps Ranch, Pascadero, CA; Nemasnap, obtained from J. Bohac (US Vegetable Laboratory, Charleston, SC); and G1805 obtained from S. P. Singh (CIAT, Cali, Colombia) and P. A. Roberts (UC Riverside, CA).

Two experiments were conducted, each with a different resistance gene system. Each experiment used Black Valentine as the susceptible genotype, together with a resistant germplasm containing one of the two resistance gene systems. Inoculated plants were used to follow nematode penetration and development, and uninoculated plants served as controls to compare plant growth with those plants inoculated with root-knot nematodes. *Gene System 1:* A susceptible common bean cultivar, Black Valentine, and a cultivar resistant to *M. incognita* race 2, Nemasnap, were used in this experiment. The four treatment combinations of Black Valentine and Nemasnap, inoculated with *M. incognita* race 2 and uninoculated, were arranged in a completely randomized design with five replications per treatment and a total of ten harvest dates. *Gene System 2:* The experimental design of the second experiment was similar; the four treatment combinations included susceptible Black Valentine and a genotype, G1805, resistant to *M. arenaria* race 1. Both experiments were conducted at the same time, and the uninoculated Black Valentine plants served as a common susceptible treatment for both experiments.

Germinated bean seeds with radicles 1 to 3 cm long were planted singly in 10-cm-diameter pots filled with a steam-sterilized sandy soil (86% sand, 2% silt, 12% clay). Seven days after planting, each bean plant was inoculated with 1,000 infective juveniles suspended in 10 ml of water, which were pipetted into three equidistant, 5-cm-deep holes around the root zone of each plant. Inoculation holes were filled with steam-sterilized soil, and pots were watered immediately to moisten the soil. Five plants of each germplasm were left uninoculated as controls for each harvest date. Plants were maintained with appropriate watering and fertilization. The experiments were carried out in a growth room maintained with a 10-hr daylength, and a mean day temperature of 25°C and night temperature of 22°C.

After 1, 2, 3, and 7 days, and every 7 days thereafter for 49 days, five inoculated and five uninoculated plants of each germplasm were harvested. The plants were cut at the soil line, and fresh and dry weights of the stems, petioles, leaf blades, and pods were recorded; areas of leaf blades also were measured. The roots were washed free of soil, blotted dry with paper towels, and weighed. Whole root systems of inoculated plants harvested 1, 2, and 3 days after inoculation were stained with acid fuchsin (Byrd *et al.*, 1983). The root systems of the uninoculated plants were dried and weighed. Root systems of plants harvested 7, 14, and 21 days after inoculation were divided into two approximately equal portions, one of which was stained; the other was dried, weighed and saved for energy analysis. Root systems of plants harvested 28, 35, 42, and 49 days after inoculation were divided into three portions; one portion was stained, one was dried and weighed, and the third was treated with 2% NaOCl to extract eggs (Hussey and Barker, 1973). All root systems were rated visually for galls using a gall rating index of 0 - 5, such that 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = more than 100 galls per root system (Taylor and Sasser, 1978).

Total numbers of nematodes in the stained portions of the root systems were recorded at each harvest date, and individuals were assigned to one of four developmental stages based on readily visible characteristics. The first developmental stage (vermiform) included vermiform, non-swollen, second-stage juveniles; the second developmental stage (swollen) included swollen, sausage-shaped, second-stage juveniles; the third developmental stage (globose) included swollen, partially globose individuals with conical tails; the final developmental stage (adult) included fully globose pre-adults and adults with or without egg masses.

Average individual fecundity (eggs per adult female) was calculated from the total number of extracted eggs per root system divided by the total number of adult females per plant. The viability of the eggs was determined as percentage of juveniles hatching from the extracted eggs in water in beakers over 10 days at 25°C. Energy analysis, using a Phillipson microbomb calorimeter (Prus, 1975), was carried out on the root, leaf blade, and stem and petiole tissues for plants harvested 21 days after inoculation, and on the root and seed tissues of plants harvested 49 days after inoculation in order to determine if infection of roots by *Meloidogyne* spp. and expression of resistance affected the distribution of energy among the plant tissues.

Both experiments were repeated once. In the second trial, the final harvest was 42 days after inoculation, since plants had senesced by 49 days after inoculation in the first trial. Data from both trials of each experiment were analyzed separately. Data were analyzed with PROC ANOVA using SAS, release 6.09 (SAS Institute, 1989).

Each day/nematode developmental stage and day/plant growth parameter was analyzed separately.

Results

Gene System 1

In the first trial, there were no differences in the total numbers of *M. incognita* race 2 in the susceptible and resistant root systems up to and including 14 days after inoculation ($P < 0.05$; Figure 2-1); a mean of 8% of the initial inoculum had penetrated the roots of Black Valentine plants, and 4% penetrated the roots of Nemasnap plants in the first 3 days after inoculation. The mean percentage of initial inoculum 14 days after inoculation was 44 % in Black Valentine roots and 35% in Nemasnap roots; beyond this time, more nematodes were present in the roots of Black Valentine plants ($P < 0.05$). By 7 days after inoculation, more nematodes had reached the swollen stage of development in the susceptible Black Valentine roots than in the resistant Nemasnap roots ($P < 0.05$). Also, beginning with day 14 and continuing through 21 days after inoculation, more nematodes reached the globose stage in the roots of Black Valentine than in the roots of Nemasnap ($P < 0.05$). More adults had developed in Black Valentine roots than in Nemasnap roots on days 21 - 49 post inoculation ($P < 0.05$ for days 21-28 and 42-49; $P < 0.10$ for day 35). At 49 days after inoculation, the presence of vermiform stages in the roots of Black Valentine indicated that development of *M. incognita* race 2 had progressed to a second generation.

In the second trial, similar rates of development of *M. incognita* race 2 were observed in Black Valentine and Nemasnap, although total populations were slightly lower (Figure 2-1). There were no differences in the numbers of nematodes penetrating both susceptible and resistant root systems up to and including 7 days after inoculation ($P < 0.05$). A mean of 6% of the initial inoculum penetrated roots of Black Valentine, and 5% penetrated roots of Nemasnap in the first 3 days after inoculation; 7 days after inoculation, 15% and 12% of initial inoculum were found in the roots of Black Valentine and Nemasnap, respectively. However, at 14 days after inoculation, more nematodes were present in the roots of Black Valentine plants ($P < 0.05$), and more of these were present in the globose stage of development compared with the nematodes in the roots of Nemasnap plants ($P < 0.05$). Again, at 21 days after inoculation, the number of nematodes present in the globose stage in Black Valentine roots was ten-fold greater than the number in Nemasnap roots ($P < 0.05$). Also at this time, some adult stages were already present in Black Valentine roots, but no adult stages were present in Nemasnap roots. In Nemasnap roots 28 days after inoculation, more nematodes were present in the swollen and globose stages than in Black Valentine roots ($P < 0.05$), where almost all the nematodes present were adults. The adult stage also was the predominant developmental stage in Black Valentine roots at 35 and 42 days after inoculation. This did not occur until 42 days after inoculation in Nemasnap roots.

When numbers of nematodes in each developmental stage were plotted as a percentage of the total numbers of nematodes in the root system, the delayed rate of development of nematodes into the adult stage in Nemasnap could be seen clearly compared to the rate of development in Black Valentine (Figure 2-2). At 28 days after inoculation, already 85% and 99% of the nematodes in the roots were present as adults in Black Valentine plants, trial 1 and 2, respectively, compared with 29% and 33% present as adults in roots of Nemasnap plants, trial 1 and 2, respectively. The interim and mature stages occurred earlier on susceptible Black Valentine roots than on Nemasnap roots. In trial 1, 21 days after inoculation, 70% of developmental stages were swollen and globose in Black Valentine roots, compared with 96% in Nemasnap roots; a similar trend was observed in trial 2. Also, the swollen stage appeared to persist longer in the resistant Nemasnap roots than in Black Valentine roots. In trial 1, 29% and 22% of nematodes in Nemasnap roots were still in the swollen stage 21 and 28 days after inoculation, respectively, compared with 14% and 2%, respectively, in Black Valentine roots; a similar trend was seen in trial 2. Although from day 35 and beyond, high percentages on both cultivars were adult females, the total numbers on which the percentages are based were much higher in Black Valentine roots (752 in trial 1 and 637 in trial 2 at 42 days after inoculation) than in Nemasnap roots (338 in trial 1 and 159 in trial 2 at 42 days after inoculation).

Galls on the root systems of Black Valentine were visible at 14 days after inoculation. At this time, the mean gall index rating was 4.8 in trial 1 and 5.0 in trial 2. Galls were first visible on the roots of Nemasnap plants at 21 days after inoculation, with mean gall index ratings of 0.8 in trial 1 and 0.2 in trial 2. The mean gall index rating on Nemasnap was highest at 42 days after inoculation at 3.4 in trial 1 and 2.0 in trial 2 (Table 2-1).

Males within and emerging from cuticles still attached to roots were present in roots of Nemasnap plants 42 days after inoculation only in the first trial; Nemasnap averaged 16 males and Black Valentine had no males per root system ($P < 0.05$). The numbers of eggs per root system and the numbers of juveniles hatching from these eggs were greater from females in Black Valentine roots than in Nemasnap roots (Table 2-2). However, there were no differences in fecundity, measured as number of eggs produced per female, of females developing in Black Valentine and Nemasnap roots (Table 2-2).

In the second trial, more males also were observed within and emerging from cuticles in Nemasnap roots than in Black Valentine roots at 28 days after inoculation ($P < 0.10$); Nemasnap averaged six males and Black Valentine had no males per root system. At 35 days after inoculation, Nemasnap had 19 males and Black Valentine had one male per root system ($P < 0.05$); at 42 days after inoculation, Nemasnap had five males and Black Valentine had no males per root system ($P < 0.10$). The numbers of eggs per root system 35 and 42 days after inoculation, and the numbers of juveniles hatching from these eggs at 42 days after inoculation were greater from females in Black Valentine roots than in Nemasnap roots ($P < 0.05$; Table 2-2). However, fecundity of females developing in Black Valentine and Nemasnap roots was similar (Table 2-2).

Plant growth in both experiments was not consistently affected by nematode inoculation as measured by total plant dry weights (Figure 2-3), leaf areas (trial 1, Table 2-3), or energy analyses (trial 1, Table 2-4). Plants under both inoculation regimes grew similarly, and were not affected by nematode inoculation.

Gene System 2

In the first trial, differences in the total numbers of *M. arenaria* race 1 in the roots of Black Valentine and resistant G1805 were significant at 14 days after inoculation, and higher levels occurred in the roots of Black Valentine plants through 49 days after inoculation ($P < 0.05$; Figure 2-4). At 7 and 14 days after inoculation, more nematodes had developed to the swollen stage in Black Valentine roots than in G1805 roots ($P < 0.05$). At days 14 - 28, higher numbers of nematodes had reached the globose stage in Black Valentine roots than in G1805 roots ($P < 0.05$). Also at days 21 - 49, there were more nematodes in the adult developmental stage in the susceptible than in the resistant roots ($P < 0.10$ for day 21; $P < 0.05$ for days 28 - 49). Second-generation vermiform juvenile nematodes were present in the roots of Black Valentine plants by 49 days after inoculation, but not in G1805 plants.

In the second trial, similar total populations and rates of development of *M. arenaria* race 1 in Black Valentine and G1805 were observed (Figure 2-4). There were no differences in the numbers of juveniles penetrating the roots up to and including 7 days after inoculation; mean percentage of initial inoculum penetrating Black Valentine roots was 12 %, and penetrating G1805 roots was 8% in the first 7 days after inoculation. After this time, population densities in the roots of Black Valentine, with the exception of 35 days after inoculation, were greater than populations in the roots of G1805 ($P < 0.05$). At 7 days after inoculation, more nematodes had developed to the swollen stage in Black Valentine roots than in G1805 roots ($P < 0.05$). The globose stage was the predominant developmental stage of nematodes present in Black Valentine roots at 14 and 21 days after inoculation. Most nematodes in G1805 roots at these times were still in the vermiform and swollen stages. Only a small number of nematodes developed to the globose stage in the roots of G1805 plants at 28 - 42 days after inoculation. At these times, most of the nematodes were in the adult developmental stage in the roots of susceptible Black Valentine plants.

The delayed rate of development of nematodes to the adult stage in G1805 compared to the rate of development in Black Valentine could be seen clearly when numbers of nematodes in each developmental stage were plotted as a percentage of the total numbers of nematodes in the root system (Figure 2-5). Relatively few *M. arenaria* race 1 nematodes in G1805 roots developed beyond the swollen developmental stage compared with those in the roots of Black Valentine, where more than 40% (trial 1) and 80% (trial 2) of the nematodes in roots already had reached the globose stage at 14 days after inoculation. Only 30% of the nematodes in roots reached the adult stage in G1805 roots at 49 days after inoculation in trial 1 and less than 10 % at 42 days after inoculation in trial 2. The actual numbers of nematodes reaching this stage were quite small compared with Black Valentine, since the total numbers on which the percentages are based are much lower in G1805.

Galls on the root systems of Black Valentine were visible at 14 days after inoculation. At this time, the mean gall index rating was 3.6 in trial 1 and 2.4 in trial 2.

The index reached 5.0 at 35 days after inoculation in trial 1, and at 28 days after inoculation in trial 2. Galls were first visible on the roots of G1805 plants at 21 days after inoculation, with a mean gall index rating of 0.8. The highest mean gall index rating on G1805 roots was 2.0 at 35 days after inoculation; even at this time the galls were very small in size, ≤ 3 mm in width. Galls were not seen on the roots of G1805 plants at any of the harvest dates in the second trial (Table 2-5).

The numbers of males within and emerging from cuticles still attached to G1805 roots were higher at 42 days (nine per root system on G1805; zero per root system on Black Valentine) and 49 days (11 per root system on G1805; zero per root system on Black Valentine) after inoculation in the first trial ($P < 0.05$); however, no male nematodes were observed in the second trial. In the first trial, the numbers of eggs per root system 35 and 42 days after inoculation and the numbers of juveniles hatching from these eggs 42 days after inoculation were greater from females in Black Valentine roots than in G1805 roots ($P < 0.05$; Table 2-6). However, there were no differences in fecundity of females developing in Black Valentine and G1805 roots (Table 2-6). In the second trial, a greater number of eggs per root system also were collected from Black Valentine plants, as compared with G1805 plants, 35 and 42 days after inoculation; a greater number of juveniles hatched from these eggs 42 days after inoculation (Table 2-6). The fecundity of females developing on Black Valentine plants was greater than that of females developing on G1805 plants (Table 2-6).

Plant growth in both experiments was not consistently affected by nematode inoculation as measured by total plant dry weights (Figure 2-6), leaf areas (trial 1, Table 2-7), or energy analyses (trial 1, Table 2-8). Plants under both inoculation regimes grew similarly, and were not affected by nematode inoculation.

Discussion

In these studies, resistance to *M. incognita* race 2 and *M. arenaria* race 1 in bean germplasm containing gene system 1 and gene system 2, respectively, was expressed by delayed nematode development rather than by differential penetration compared with a susceptible plant. Penetration of *Meloidogyne* spp. occurred in similar numbers into roots of the resistant and susceptible genotypes, indicating that root barriers to penetration do not play a role in resistance of these germplasms. Penetration of equal numbers of root-knot nematode juveniles into roots of common bean plants also has been reported by Barrons (1939) and by Fassuliotis *et al.* (1970). Similar observations have been made in resistant and susceptible corn (Windham and Williams, 1994), cotton (Creech *et al.*, 1995), soybean (Herman *et al.*, 1991), and tobacco (Schneider, 1991).

The populations of *M. incognita* and *M. arenaria* in Black Valentine roots increased over time, indicating that the nematodes continued to enter roots of the susceptible genotype over an extended period of time. Juveniles of *M. incognita* also continued to enter the roots of the resistant Nemasnap plants over time; however, this occurred at a much reduced rate compared to the susceptible Black Valentine plants. As the population of *M. arenaria* in the roots of G1805 remained at a similar or lower level than the number that had penetrated by 3 days after inoculation, it is possible that an inability of some of the juveniles to establish feeding sites led to their emigration from the roots, as observed in other plant species (Herman *et al.*, 1991; Schneider, 1991).

Delayed development of *M. incognita* race 2 in Nemasnap and *M. arenaria* race 1 in G1805, as compared with the development of these nematodes in susceptible Black Valentine plants, indicates that a post-infectional mechanism is operating to retard nematode development. Conditions adverse to nematode development in resistant plants, such as unavailability of nutrients in the root cells, presence of preformed nematotoxic chemicals, induced production of phytoalexins, or a combination of these mechanisms (J. S. Huang, 1985; Kaplan *et al*., 1980), may be responsible for the slowed development of root-knot nematodes in the roots of resistant common bean plants. An increased number of males and reduced egg production in the roots of resistant plants also were observed in our studies. More information on the biochemical processes involved in post-infectional resistance is necessary for identifying effective resistance mechanisms.

Work by Melakeberhan *et al*. (1985a; 1985b) demonstrated that *Meloidogyne incognita* infection of susceptible bean plants has a significant effect on the growth, physiology, and nutrient content of the plant. They reported that in nematode-infected plants, most growth parameters, such as leaf area and shoot, root and total plant weight, generally decreased with increasing nematode inoculum and duration of infection. Overall plant weight increased over time; however, the increase was double for nematode-infected plants, as compared with five times for uninoculated plants (Melakeberhan *et al*. 1985b). They also observed that nematode infection reduced the photosynthetic rate, chlorophyll content, and total content of nutrient elements per plant, which resulted in slower plant growth as compared with uninfected plants (Melakeberhan *et al*. 1985a). In the present studies, the growth parameters of plants maintained under the growth room conditions increased over time; however, differences between inoculated and uninoculated plants of the susceptible or resistant germplasms, as reported by Melakeberhan *et al*. (1985a; 1985b), were not observed. This may be due to the relatively low initial inoculum levels used in this study compared with those used by Melakeberhan *et al*. (H. Melakeberhan, personal communication).

Feeding of root-knot nematodes has been shown to be more expensive in energy costs to susceptible plants than to resistant plants (Melakeberhan and Ferris, 1989; Powers and McSorley, 1993). Energy analyses of leaf, stem and petiole, root and seed tissues of susceptible Black Valentine plants in the present studies did not indicate that *Meloidogyne* spp. infection was costly in energy terms, either per milligram of tissue, per total tissue, or per total plant weight, to the susceptible or resistant genotypes, as compared to uninfected plants. These data are supported by the plant growth parameters showing few differences between inoculated and uninoculated plants of the susceptible and resistant germplasms. Because there were no differences in the energy content between tissues of the inoculated and uninoculated resistant genotypes, these data do not support my hypothesis that resistance mechanisms are energy-requiring processes re-allocating energy away from vegetative growth or fruit production.

The resistance response against *M. arenaria* race 1 in bean germplasm G1805 containing gene system 2 appears to be more restrictive than the response against *M. incognita* race 2 observed in Nemasnap containing gene system 1. Very few nematodes in G1805 roots developed beyond the swollen stage. I suggest that this is due to the failure of giant cell development. Since areas of discolored and somewhat collapsed tissues were observed frequently around vermiform and swollen nematodes in these roots, I suggest that a hypersensitive reaction occurs which inhibits normal or further giant cell development, resulting in arrested nematode development. The small number of nematodes that did advance to globose and adult stages were present in small root galls, indicating that some giant cell initiation and development had occurred normally in a few cases.

In Nemasnap roots, development of *M. incognita* race 2 was delayed compared to development in Black Valentine roots, and fewer nematodes advanced to the mature stages. However, in comparison to *M. arenaria* race 1 in G1805 roots, discolored and collapsed tissues were not seen around nematodes in Nemasnap roots, and a larger number of nematodes developed to the globose stage. It appears that once nematodes have reached the globose stage in the roots of both resistant germplasms, development to adults will occur. A similar observation has been reported by Jenkins *et al.* (1995) with *M. incognita* and resistant cotton genotypes. The critical stage in Nemasnap seems to be at 21 - 28 days after inoculation, when development from swollen to globose stages occurs. In G1805, development to globose stages does not occur until 28 - 35 days after inoculation, at which time the numbers of *M. arenaria* race 1 decline somewhat, probably due to the death and decay of the nematodes that cannot continue development.

I speculate that the single dominant gene controlling resistance in plants containing gene system 2 is effective early in the nematode-plant interaction, and is expressed as a hypersensitive reaction in response to the attempts of nematodes to establish feeding sites. The interaction of the two or more recessive genes controlling resistance in gene system 1 plants was reported also to result in a hypersensitive reaction resulting in giant cell dissolution early in the nematode-plant interaction (Fassuliotis *et al.*, 1970). My studies indicate that this resistance is less efficient than that expressed by the single dominant gene in germplasm containing gene system 2.

Table 2-1. Gall index rating of root systems of Black Valentine and Nemasnap bean plants inoculated with *Meloidogyne incognita* race 2.

	Gall index rating, trial 1		Gall index rating, trial 2	
Harvest day	Black Valentine	Nemasnap	Black Valentine	Nemasnap
14	4.8	0**	5.0	0**
21	5.0	0.8**	5.0	0.2**
28	5.0	1.6**	5.0	0.6**
35	5.0	2.6**	5.0	0**
42	5.0	3.4**	5.0	2.0**
49	5.0	3.6**	-	-

Data are means of five replications.

** Data differ from number on Black Valentine ($P < 0.05$).

Table 2-2. Numbers of eggs of *Meloidogyne incognita* race 2 extracted from the root systems of Black Valentine and Nemasnap bean plants, juveniles hatching from the eggs after 10 days, and eggs per female.

	Eggs/root system		Hatched juveniles		Eggs/female	
Harvest day	Black Valentine	Nemasnap	Black Valentine	Nemasnap	Black Valentine	Nemasnap
Trial 1						
28	201	0	0	0	0	0
35	10,200	1,740[**]	646	104[†]	40	12
42	16,200	4,920	3,420	889[†]	23	13
Trial 2						
35	3,470	125[*]	56	0	8	1
42	23,300	3,560[**]	1,760	131[*]	64	27

Data are means of five replications.

[*,**] Log$_{10}$-transformed data differ from number on Black Valentine at $P < 0.05$ and $P < 0.01$, respectively.

[†] Log$_{10}$-transformed data differ from number on Black Valentine at $P < 0.10$.

Table 2-3. Leaf areas of Black Valentine and Nemasnap bean plants uninoculated (NN) or inoculated with *Meloidogyne incognita* race 2 (*Mi*2).

	Leaf area (cm^2)			
	Black Valentine		Nemasnap	
Harvest day	NN	*Mi*2	NN	*Mi*2
7	153.71	86.50	95.57	109.13
14	164.01	235.78	234.41	173.65
21	304.95	382.59	362.89	445.83
28	546.50	556.13	610.68	455.52
35	536.88	470.83	498.70	653.22
42	515.66	570.63	650.23	547.38
49	308.67	318.80	87.46	216.67

Data are means of five replications.

No significant differences ($P < 0.05$) were observed between leaf areas of plants uninoculated or inoculated with *Meloidogyne incognita* race 2.

Table 2-4. Energy content of plant tissues of Black Valentine and Nemasnap bean plants uninoculated (NN) or inoculated with *Meloidogyne incognita* race 2 (*Mi*2).

	Energy content 21 days after inoculation (joules/mg)				Energy content 49 days after inoculation (joules/mg)			
Plant	Black Val.		Nemasnap		Black Val.		Nemasnap	
tissue	NN	*Mi*2	NN	*Mi*2	NN	*Mi*2	NN	*Mi*2
Leaf blade	20.41	17.47	19.44	19.23	-	-	-	-
Stem & petiole	15.75	15.13	14.58	14.62	-	-	-	-
Root	11.15	12.36	10.69	11.19	13.87	9.97***	12.95	12.49
Seed	-	-	-	-	15.75	17.35	16.47	16.30

Data are means of three replications.

*** Data differ from uninoculated Black Valentine at $P < 0.01$.

Table 2-5. Gall index rating of root systems of Black Valentine and G1805 bean plants inoculated with *Meloidogyne arenaria* race 1.

	Gall index rating, trial 1		Gall index rating, trial 2	
Harvest day	Black Valentine	G1805	Black Valentine	G1805
14	3.6	0**	2.4	0**
21	4.8	0.8**	2.6	0**
28	4.2	0**	5.0	0**
35	5.0	2.0**	5.0	0**
42	5.0	2.0**	5.0	0**
49	5.0	2.0**	-	-

Data are means of five replications.

** Data differ from number on Black Valentine ($P < 0.05$).

Table 2-6. Numbers of eggs of *Meloidogyne arenaria* race 1 extracted from the root systems of Black Valentine and G1805 bean plants, juveniles hatching from the eggs after 10 days, and eggs per female.

	Eggs/root system		Hatched juveniles		Eggs/female	
Harvest day	Black Valentine	G1805	Black Valentine	G1805	Black Valentine	G1805
Trial 1						
28	54	0	0	0	0	0
35	1,780	594**	16	0	5	49
42	11,000	90**	4,080	0**	11	7
Trial 2						
35	3,630	13*	41	0	14	0*
42	29,700	29*	3,780	0*	64	3†

Data are means of five replications.

*,** Log$_{10}$-transformed data differ from number on Black Valentine at $P < 0.05$ and $P < 0.01$, respectively.

† Log$_{10}$-transformed data differ from number on Black Valentine at $P < 0.10$.

Table 2-7. Leaf areas of Black Valentine and G1805 bean plants uninoculated (NN) or inoculated with *Meloidogyne arenaria* race 1 (*Ma*1).

	Leaf area (cm^2)			
	Black Valentine		G1805	
Harvest day	NN	*Ma*1	NN	*Ma*1
7	153.71	143.61	105.80	99.73
14	164.01	196.70	196.03	136.86
21	304.95	361.26	293.46	355.11
28	546.50	570.69	481.33	356.69
35	536.88	547.41	494.93	539.76
42	515.66	581.58	621.80	691.02
49	308.67	359.03	371.58	199.10

Data are means of five replications.

No significant differences ($P < 0.05$) were observed between leaf areas of plants uninoculated or inoculated with *Meloidogyne arenaria* race 1.

Table 2-8. Energy content of plant tissues of Black Valentine and G1805 bean plants uninoculated (NN) or inoculated with *Meloidogyne arenaria* race 1 (*Ma*1).

	Energy content 21 days after inoculation (joules/mg)				Energy content 49 days after inoculation (joules/mg)			
Plant	Black Val.		G1805		Black Val.		G1805	
tissue	NN	*Ma*1	NN	*Ma*1	NN	*Ma*1	NN	*Ma*1
Leaf blade	20.41	19.02***	18.39	18.06	-	-	-	-
Stem & petiole	15.75	15.04	15.38	15.34	-	-	-	-
Root	11.15	11.65	11.65	11.02	13.87	18.60	12.70	11.77
Seed	-	-	-	-	15.75	16.80	12.26	16.76

Data are means of three replications.

*** Data differ from uninoculated Black Valentine at $P < 0.01$.

Figure 2-1. Penetration and development of *Meloidogyne incognita* race 2 in susceptible Black Valentine and resistant Nemasnap common bean germplasm (gene system 1, trials 1 and 2).
Data are means of five replications.
At each harvest day within each trial, asterisks (*) indicate significantly higher numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in roots of the other germplasm ($P < 0.05$).

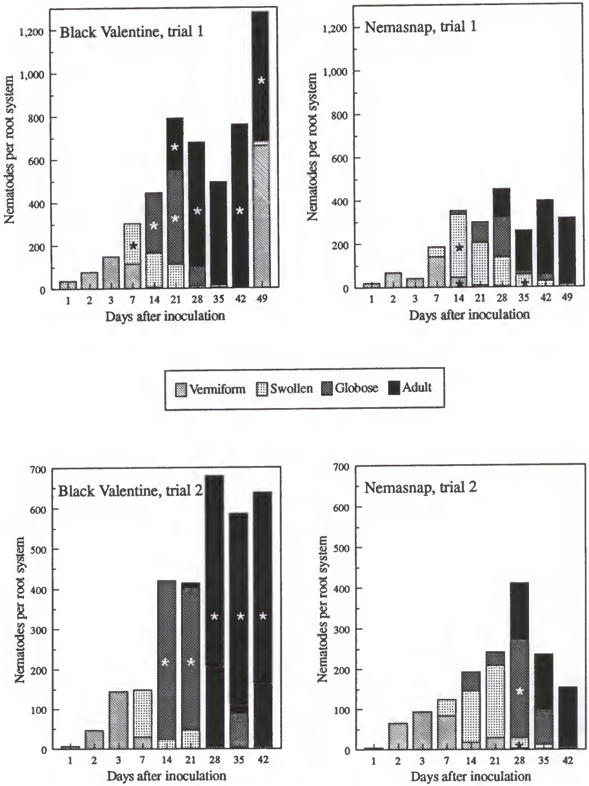

Black Valentine, trial 1
Nemasnap, trial 1
Black Valentine, trial 2
Nemasnap, trial 2
Nematodes per root system
Days after inoculation
1 2 3 7 14 21 28 35 42 49
0 200 400 600 800 1,000 1,200
0 100 200 300 400 500 600 700
▨ Vermiform
▨ Swollen
▨ Globose
■ Adult
*

Figure 2-2. Percentage of the population of *Meloidogyne incognita* race 2 in four developmental stages in root systems of susceptible Black Valentine and resistant Nemasnap common bean germplasm (gene system 1, trials 1 and 2).
Data are means of five replications.

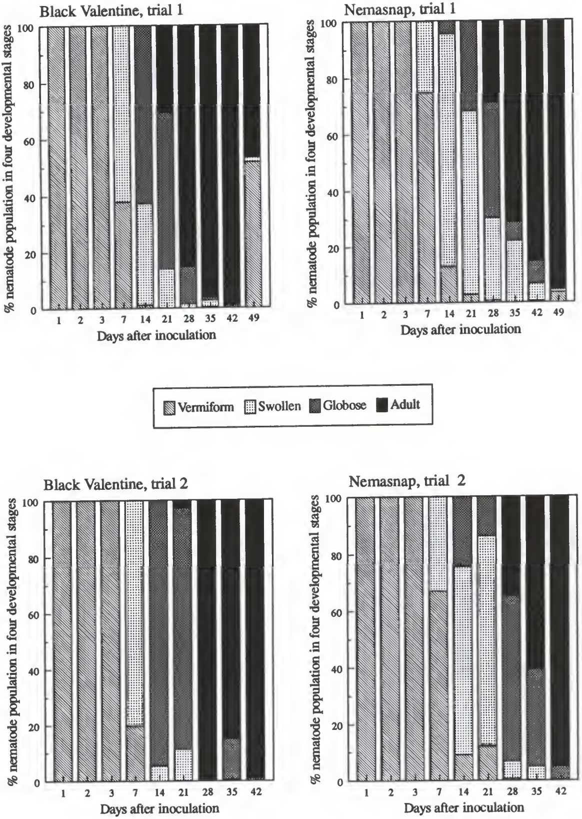

Black Valentine, trial 1
Nemasnap, trial 1
Black Valentine, trial 2
Nemasnap, trial 2
% nematode population in four developmental stages
0
20
40
60
80
100
1 2 3 7 14 21 28 35 42 49
1 2 3 7 14 21 28 35 42
Days after inoculation
Vermiform
Swollen
Globose
Adult

Figure 2-3. Total plant dry weights of Black Valentine (BV) and Nemasnap (NS) uninoculated (NN) or inoculated with *Meloidogyne incognita* race 2 (*Mi*2) (gene system 1, trials 1 and 2).
Data are means of five replications.

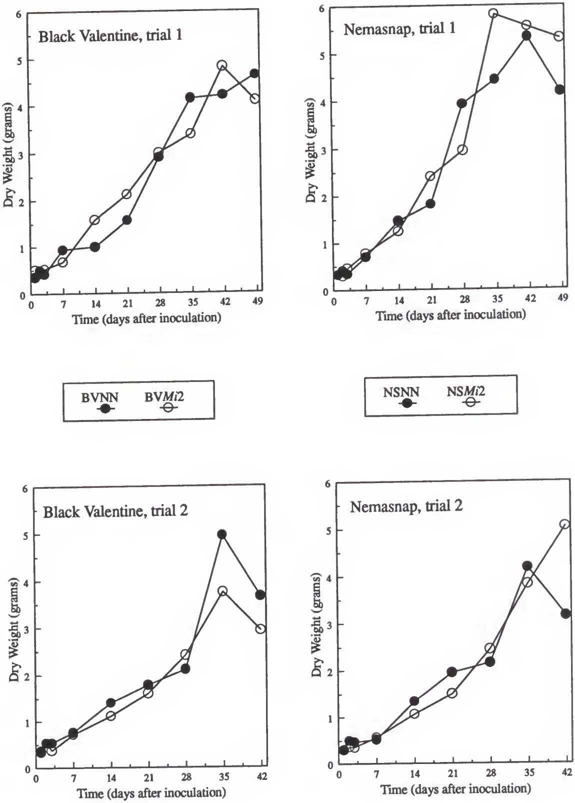

Black Valentine, trial 1
Nemasnap, trial 1
Black Valentine, trial 2
Nemasnap, trial 2
Dry Weight (grams)
Time (days after inoculation)
0
1
2
3
4
5
6
0
7
14
21
28
35
42
49
BVNN
BV*Mi*2
NSNN
NS*Mi*2

Figure 2-4. Penetration and development of *Meloidogyne arenaria* race 1 on susceptible Black Valentine and resistant G1805 common bean germplasm (gene system 2, trials 1 and 2).

Data are means of five replications.

At each harvest day within each trial, asterisks (*) indicate significantly higher numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in roots of the other germplasm ($P < 0.05$).

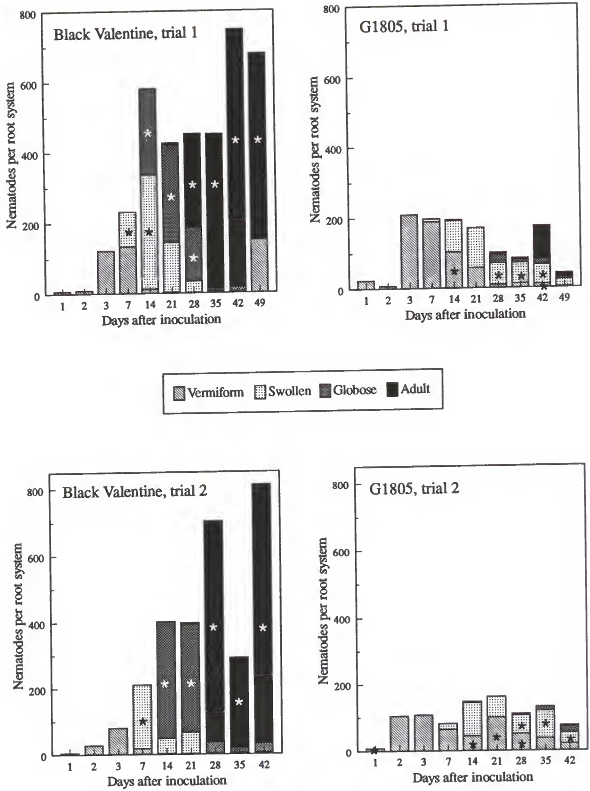

Black Valentine, trial 1
G1805, trial 1
Black Valentine, trial 2
G1805, trial 2
Nematodes per root system
Days after inoculation
0
200
400
600
800
1
2
3
7
14
21
28
35
42
49
Vermiform
Swollen
Globose
Adult
*

Figure 2-5. Percentage of the population of *Meloidogyne arenaria* race 1 in four developmental stages in root systems of susceptible Black Valentine and resistant G1805 common bean germplasm (gene system 2, trials 1 and 2).
Data are means of five replications.

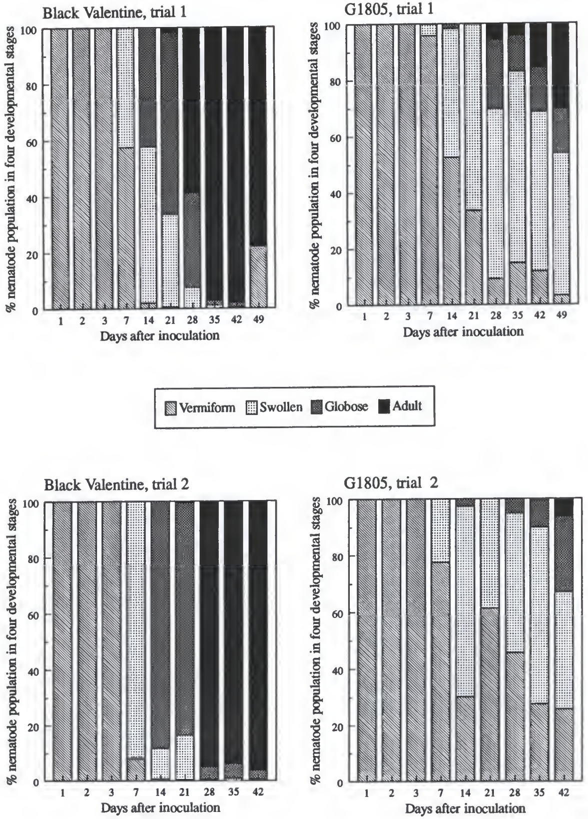
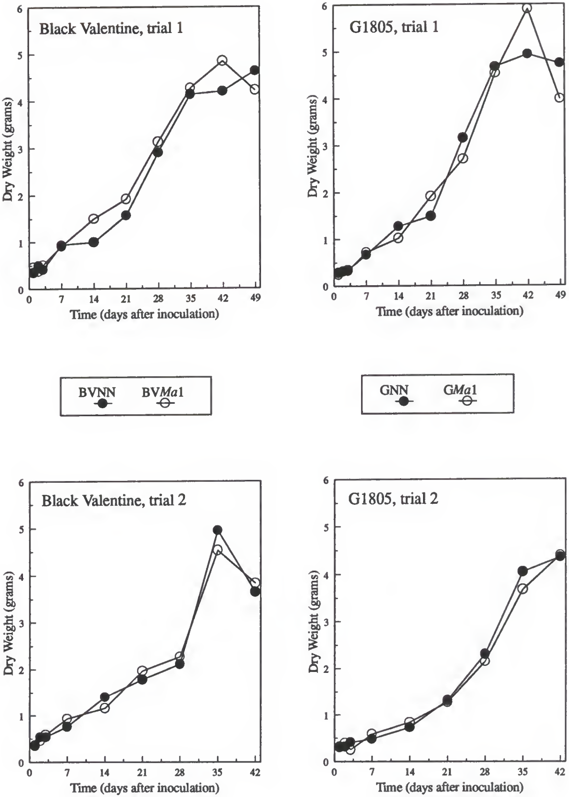

Black Valentine, trial 1
G1805, trial 1
Black Valentine, trial 2
G1805, trial 2
% nematode population in four developmental stages
0
20
40
60
80
100
1
2
3
7
14
21
28
35
42
49
Days after inoculation
▨ Vermiform
▨ Swollen
▨ Globose
■ Adult

Figure 2-6. Total plant dry weights of Black Valentine (BV) and G1805 (G) uninoculated (NN) or inoculated with *Meloidogyne arenaria* race 1 (*Ma*1) (gene system 2, trials 1 and 2).
Data are means of five replications.


Black Valentine, trial 1
G1805, trial 1
BVNN
BVMa1
GNN
GMa1
Black Valentine, trial 2
G1805, trial 2
Dry Weight (grams)
Time (days after inoculation)
0
1
2
3
4
5
6
7
14
21
28
35
42
49

CHAPTER 3

EFFECTS OF TEMPERATURE ON RESISTANCE IN NEMASNAP AND G1805 BEAN GERMPLASM AND ON DEVELOPMENT OF *MELOIDOGYNE INCOGNITA* RACE 2 AND *MELOIDOGYNE ARENARIA* RACE 1

Introduction

Over 77% of the world production of common bean (*Phaseolus vulgaris* L.) occurs in the tropical developing countries of Latin America and Africa (Pachico, 1989). The majority of bean production in these areas occurs during cool, dry seasons or at high altitudes where temperatures range from 16°C to 24°C, which are close to the optimum temperatures for *P. vulgaris* growth (Allen *et al.*, 1989; van Schoonhoven and Voysest, 1989). However, soil temperatures higher than the optimum range often occur in bean-growing regions throughout the world. Elevated soil temperatures can severely increase the stress on bean plants. In these situations, the use of cultivars with heat-stable resistance to *Meloidogyne* spp. may be critical for the management of root-knot nematode problems.

The effects of high soil temperature on the expression of resistance to root-knot nematodes have been reported for several crop plants (Ammati *et al.,* 1986; Carter, 1982, Dropkin, 1969), including common beans (Fassuliotis *et al.,* 1970; Irizarry *et al.*, 1971; Mullin *et al.,* 1991a; Omwega *et al.*, 1990a). In general, resistance to nematodes decreases as temperature increases (Carter, 1982, Dropkin, 1969; Fassuliotis *et al.,* 1970). Fassuliotis *et al.* (1970) found that roots of both a susceptible cultivar, Black Valentine, and a resistant breeding line, B3864, showed light galling at a lower temperature (16°C), and that more galls were visible as temperatures increased to 21°C and 28°C. Production of females and egg masses also increased with the increase in temperature, but the numbers of mature females that developed in the roots of the resistant lines at the higher temperatures were still lower than those in the susceptible cultivar. Also based on the number of mature females, bean lines Alabama #1 and PI 165435 lost their resistance to *M. incognita* and *M. hapla*, but not *M. arenaria*, when soil temperature increased from 25°C to 30°C (Irizarry *et al.* 1971). Omwega *et al.* (1990a) also found that resistance to *M. incognita* race 2 was lost at 30°C in Alabama #1 and PI 165435, but remained partially effective in PI 165426. Resistance to *M. incognita* race 1 and *M. javanica* in other bean lines was stable at temperatures of 24°C to 30°C; however, *M. javanica* populations increased as temperatures increased from 26°C to 30°C. Mullin *et al.* (1991a) reported that bean line A211 was resistant to *M. incognita* at 16°C and 22°C, but susceptible at 24°C and above. They found that short incubation times (< 16 days) of inoculated plants at 30°C, before low temperature incubation, resulted in loss of resistance as measured by nematode reproduction and root galling. Incubation of inoculated plants at 21°C for > 16 days, before high temperature incubation, resulted in retention of resistance expression during a high temperature treatment.

Previous studies have reported the effect of temperature on resistance expression largely in terms of gall ratings, numbers of adults, and reproduction measured as egg mass and total egg production. Previous workers have not studied the effect of temperature on resistance expression either prior to or during the process of nematode penetration and nematode development in resistant germplasm. The focus of the current research was to understand the effect of temperature on the relationship between the developmental behavior of *Meloidogyne* spp. and resistance in bean plants in order to predict how resistant germplasm would react under high soil temperature stress. The objective of this study was to evaluate the effect of temperature on resistance expression in *P. vulgaris* germplasm; specifically, to evaluate if pre-inoculation conditioning temperatures modify resistance before the pressure of nematode infection, and to evaluate the effect of post-inoculation incubation temperatures on resistance expression.

Materials and Methods

All experiments were conducted at the University of Florida in Gainesville, Florida during 1995. The protocol for all experiments was similar. The origin and maintenance of these populations of *M. incognita* race 2 and *M. arenaria* race 1 were the same as described previously (Materials and Methods, Chapter 2).

Gene System 1 and Gene System 2

Separate experiments were conducted, each evaluating various temperature combinations with a different resistance gene system. Each experiment used Black Valentine as the susceptible genotype, together with a resistant germplasm containing one of the two resistance gene systems. *Gene System 1:* A susceptible common bean cultivar, Black Valentine, and Nemasnap, a cultivar resistant to *M. incognita* race 2, were used in this experiment. Treatment combinations on Black Valentine or Nemasnap, all inoculated with *M. incognita* race 2, were arranged in a completely randomized design with four replications per treatment and a total of seven harvest dates. *Gene System 2:* The experimental design of the second experiment was similar. Treatment combinations were imposed on susceptible Black Valentine or germplasm G1805, resistant to *M. arenaria* race 1.

All experiments were conducted in growth chambers with 10 hours of light and 14 hours of dark. The temperatures used in all experiments as the pre-inoculation conditioning temperature or the post-inoculation incubation temperature were 24°C and 28°C; 24°C is within the range at which resistance is stable, and 28°C is within the range at which resistance is reported to be modified, but still sufficiently low for bean plant growth not to be too heat-stressed (Omwega *et al.*, 1990a). Therefore, the temperature regimes established were 24/24°C, 24/28°C, 28/24°C, and 28/28°C, with the first temperature being the pre-inoculation temperature and the latter being the post-inoculation temperature. All plants were grown for a total of 99 accumulated heat units (degree days over 10°C base) before inoculation, to ensure that all the plants were of similar physiological age at the time of inoculation. All chambers were maintained at a constant relative humidity level of 75%.

Germinated bean seeds with radicles 1 to 3 cm long were planted singly in a CYG Seed Growth Pouch (Mega International, Minneapolis, MN) and watered with approximately 15 ml of water. Randomly chosen pouches were inserted in pairs in manila folders, for vertical support, and placed in the growth chambers set at the four temperature regimes. Each bean plant was inoculated by pipetting a 2- to 2.5-ml water suspension, containing 400 infective juveniles, over the roots of the seedling in the pouch. The pouches were returned to the growth chambers and kept horizontal for 24 hours to ensure the inoculum remained in the root zone before returning the pouches to an upright position for the remainder of the experiment.

At 1, 2, 3, 7, 14, 21, and 28 days after inoculation, four plants of each germplasm were harvested. The plants were cut at the root line and fresh weights of the top and root system were measured. The roots were stained with acid fuchsin (Byrd *et al.*, 1983). Total numbers of nematodes in the stained root systems were recorded at each harvest date, and individuals were assigned to one of four developmental stages based on readily visible characteristics. The first developmental stage (vermiform) included vermiform, non-swollen, second-stage juveniles; the second developmental stage (swollen) included swollen, sausage-shaped, second-stage juveniles; the third developmental stage (globose) included swollen, partially globose individuals with conical tails; the final developmental stage (adult) included fully globose pre-adults and adults with or without egg masses.

Both experiments were repeated once. Data from both trials of each experiment were analyzed separately. Data were analyzed by analysis of variance using the PROC ANOVA procedure from SAS, release 6.09 (SAS Institute, 1989). In order to determine the effects of the pre- and post-inoculation temperatures on nematode penetration and development, data from the respective temperature regimes were pooled for analysis. All the replications for each temperature regime were within one chamber, and thus it is not possible to separate the effects of temperature from positional effects. However, due to the stability and consistency of the temperature and humidity levels in all the growth chambers used in these experiments, positional effects are not considered to have had significant influence on the results obtained. Thus the discussion is confined to the effects of temperature only.

High Temperature Experiments 1 and 2

In order to examine the effects of high temperature on resistance expression of gene system 1 and gene system 2, two small additional experiments were conducted in growth chambers set at 30°C and 32°C. Each experiment used Black Valentine as the susceptible genotype, together with either Nemasnap and *M. incognita* race 2 (gene system 1; experiment 1) or G1805 and *M. arenaria* race 1 (gene system 2; experiment 2). The treatment combinations were arranged in completely randomized designs with three replications per treatment and two harvest times, 13 and 22 days after inoculation. All plants were grown for five days before inoculation with 400 second-stage juveniles of either *M. incognita* race 2 or *M. arenaria* race 1. Plants were grown in seed growth pouches. Plant maintenance, inoculation, harvest, data collection procedures, and data analysis were conducted as described earlier in this section

Results

Gene System 1

For both Black Valentine and Nemasnap, the effects of the four temperature regimes on *Meloidogyne incognita* race 2 are presented as total nematode populations (Figure 3-1), and the effects of the pre- and post inoculation temperatures are presented as the distribution of nematodes among the developmental stages (Figures 3-2 to 3-5).

The total populations of *M. incognita* race 2 present in roots of susceptible Black Valentine plants were similar among the four temperature regimes (Figure 3-1). Differences were observed in trial 1 only at 7 days after inoculation, when the population in plants grown at the 24/24°C temperature regime was smaller than the populations in plants grown at the other temperature regimes ($P < 0.05$); at 28 days after inoculation in trial 2, total populations were greater in plants grown at the lower post-inoculation temperature of 24°C than were total populations in plants grown at the post-inoculation temperature of 28°C ($P < 0.05$). By 14 days after inoculation in trials 1 and 2, the populations within the roots of Black Valentine plants grown at all temperature regimes were approximately 50% of the initial inoculum level (Figure 3-1).

The total populations of *M. incognita* race 2 within the roots of resistant Nemasnap were more variable among the four temperature regimes (Figure 3-1). At 1 day after inoculation in trials 1 and 2, more nematodes were present in the roots of plants grown at the 28/28°C temperature regime than in roots of plants grown at the other temperature regimes ($P < 0.05$). At 14 and 21 days after inoculation, the populations of *M. incognita* race 2 in plants grown at the four temperature regimes were not similar (trial 1, $P < 0.05$; trial 2, $P < 0.10$); however, these differences in total population numbers were not consistent among the four temperature regimes. By 2 days after inoculation in both trials 1 and 2, the populations of *M. incognita* race 2 had peaked to maximum levels of less than 40% of the initial inoculum. Beyond this time, the populations of nematodes within the roots did not increase further. Only 25% of the initial inoculum were within the roots of Nemasnap plants at the end of the experiment (Figure 3-1).

Overall, fewer nematodes of *M. incognita* race 2 were observed in the roots of resistant Nemasnap plants than in roots of susceptible Black Valentine plants. At 28 days after inoculation, the mean total numbers of nematodes, pooled over all four temperature regimes, were higher in Black Valentine (222 in trial 1, and 233 in trial 2), than in Nemasnap (72 in trial 1, and 116 in trial 2) ($P < 0.05$; Figure 3-1).

At 28 days after inoculation, a second generation of vermiform second-stage juveniles had penetrated the roots of susceptible Black Valentine plants grown at the higher post-inoculation temperature of 28°C. Roots of Black Valentine plants grown at the lower post-inoculation temperature of 24°C had not been penetrated by a second generation of juveniles by this time (Figures 3-4 and 3-5). The population data for second-generation vermiform juveniles at 28 days after inoculation were not plotted, in order to emphasize that the total numbers of first-generation nematodes in Black Valentine roots were similar in plants grown under all temperature regimes (Figure 3-1). The numbers of second-generation vermiform juveniles present in plants grown at 28/28°C were 278 (trial 1) and 261 (trial 2); the numbers in plants at 24/28°C were lower, with 30 in trial 1 and 98 in trial 2 (data not shown).

From 14 to 28 days after inoculation in trials 1 and 2, males of *M. incognita* race 2 were observed in the roots of both Black Valentine and Nemasnap plants at all temperature regimes. Pre- and post inoculation temperatures of 24°C and 28°C had no consistent effect on the numbers of males in the susceptible or the resistant plants (Table 3-1).

Pre-inoculation conditioning temperatures affected the populations of *M. incognita* race 2 in roots of Black Valentine and Nemasnap plants. However, these effects were not consistent between the two temperatures in either germplasm (Figures 3-2 and 3-3). In trials 1 and 2, pre-inoculation conditioning temperatures had no effect on the numbers of nematodes penetrating the roots of Black Valentine plants during the first three days after inoculation ($P < 0.05$; Figures 3-2 and 3-3). The total numbers of nematodes in the roots of Black Valentine plants at all harvest times were similar between the two temperatures ($P < 0.05$) in both trials 1 and 2. Development in trial 2, however, was more rapid in plants conditioned at the higher pre-inoculation temperature, demonstrated by the larger number of nematodes in the globose stage at 7 days after inoculation at 28°C than at 24°C ($P < 0.05$), and by the larger number of nematodes remaining in the swollen stage at 14 days after inoculation at 24°C than at 28°C ($P < 0.05$; Figure 3-3).

Pre-inoculation conditioning temperatures had little effect on nematode penetration and development in the roots of Nemasnap plants. The only significant effects were at 1 day after inoculation in trial 1, when 50% more nematodes had penetrated the roots of plants conditioned at the higher pre-inoculation temperature than at the lower temperature ($P < 0.05$; Figure 3-2), and in trial 2 at 28 days after inoculation, when 27% more nematodes remained in the globose stage at 24°C than at 28°C ($P < 0.05$; Figure 3-3).

Post-inoculation incubation temperatures had greater effects than pre-inoculation conditioning temperatures on the development of *M. incognita* race 2 in the roots of both susceptible Black Valentine and resistant Nemasnap plants. However, in trials 1 and 2 during the first 3 days after inoculation, the numbers of vermiform nematodes penetrating roots of Black Valentine plants were not influenced by post-inoculation temperatures (Figures 3-4 and 3-5). At 7 days after inoculation in both trials 1 and 2, nematode development had progressed to the swollen stage in Black

Valentine roots incubated at both post-inoculation temperatures; however, at the higher temperature at this time, significantly more (98% in trial 1, and 82% in trial 2) nematodes had developed to the globose stage than at the lower temperature ($P < 0.05$). In trial 2 also at this time, 43% more nematodes remained in the swollen stage at 24°C than at 28°C ($P < 0.05$). At 14 days after inoculation in both trials, 60% more nematodes remained in the globose stage in roots of Black Valentine plants incubated at 24°C than in plants incubated at 28°C; also, more adults were present in plants incubated at 28°C (97% in trial 1, and 72% in trial 2) than at 24°C ($P < 0.05$). At 21 days after inoculation, the distribution of the *M. incognita* race 2 populations among the four developmental stages was similar in roots of plants incubated at both temperatures. There was an exception to this similarity in trial 1; 69% more globose stages were present at 24°C than at 28°C ($P < 0.05$). At 28 days after inoculation, more nematodes (87% in trial 1, and 50% in trial 2) remained in the globose stage in roots incubated at 24°C than in roots incubated at 28°C ($P < 0.05$). Large numbers of adults were present in the roots of plants incubated at both temperatures; in trial 2, 30% more adults were found at 24°C than at 28°C ($P < 0.05$). This occurred because at this time in trials 1 and 2, roots of Black Valentine plants incubated at 28°C were penetrated by a second generation of vermiform juveniles; this did not occur in roots of plants incubated at 24°C.

Both penetration into and development of *M. incognita* race 2 in the roots of resistant Nemasnap were affected by post-inoculation incubation temperatures (Figures 3-4 and 3-5). However, the influence of the post-inoculation temperatures on numbers of vermiform juveniles penetrating Nemasnap roots in the first three days after inoculation was not consistent. In trial 2 at 1 day after inoculation, 51% more juveniles penetrated roots incubated at 28°C than at 24°C ($P < 0.05$), whereas, in trial 1 at 3 days after inoculation, 36% more juveniles penetrated roots incubated at 24°C than at 28°C ($P < 0.05$; Figures 3-4 and 3-5).

The effects of the post-inoculation incubation temperatures on the development of *M. incognita* race 2 in Nemasnap plants were more pronounced. At 7 days after inoculation in trials 1 and 2, a mean of 50% more nematodes were present in the vermiform stage in roots of plants incubated at the lower temperature than in roots of plants incubated at the higher temperature ($P < 0.05$; Figures 3-4 and 3-5). Also at this time, a mean of 50% more nematodes were present in the globose stage in plants incubated at 28°C than in plants incubated at 24°C (trial 1, $P < 0.05$; trial 2, $P < 0.10$). At 14 days after inoculation, adults had developed in roots of Nemasnap plants incubated at 28°C, but were absent from roots incubated at 24°C. There were significantly more adults at 28°C than at 24°C ($P < 0.05$) at 21 days after inoculation (71% in trial 1, and 63% in trial 2). In trial 1 at 21 and 28 days after inoculation, 68% more nematodes still remained in the globose stage in roots incubated at 24°C compared with the numbers of globose nematodes in roots incubated at 28°C ($P < 0.05$). At the higher temperature, at 28 days after inoculation, 80% more nematodes were present in the vermiform stage in trial 1 and 35% more were present in the swollen stage in trial 2 than at the lower temperature ($P < 0.05$).

Gene System 2

Total populations of *Meloidogyne arenaria* race 1 within the roots of susceptible Black Valentine plants, at all the harvest times, were not different among the four temperature regimes ($P < 0.05$) in both trial 1 and in trial 2 (Figure 3-6). By 14 days after inoculation, approximately 50% of the initial inoculum had entered and developed in root systems of the susceptible plants grown under all four temperature regimes (Figure 3-6).

Total populations of *M. arenaria* race 1 present in the roots of resistant G1805 plants also were similar among the four temperature regimes (Figure 3-6). Differences between numbers of nematodes in plants grown at the 24/24°C and the 28/28°C temperature regimes were observed in trial 1 only at 1 day after inoculation ($P < 0.05$), and in trial 2 only at 7 days after inoculation, when plants grown under the 28/24°C regime had a higher population of *M. arenaria* race 1 than plants grown under the other temperature regimes. By 3 days after inoculation in trial 1 and 2 days after inoculation in trial 2, numbers of *M. arenaria* race 1 had reached their maximum levels; in both trials, less than 30% of the initial inoculum had entered in the roots of the resistant G1805 plants grown under all temperature regimes. After these times, the numbers of nematodes within the root systems began to decline, reaching levels below 10 % of the initial inoculum level.

Overall, the populations of *M. arenaria* race 1 in the roots of resistant G1805 plants were lower than the populations in the roots of Black Valentine plants. At 28 days after inoculation, the mean total numbers of nematodes, pooled over all four temperature regimes, were higher in Black Valentine (219 in trial 1, and 199 in trial 2), than in G1805 (52 in trial 1, and 34 in trial 2) ($P < 0.05$; Figure 3-6).

At 28 days after inoculation, a second generation of vermiform second-stage juveniles had penetrated the roots of susceptible Black Valentine plants grown at the higher post-inoculation temperature of 28°C; these were absent from the roots of Black Valentine plants grown under the lower post-inoculation temperature of 24°C (Figures 3-9 and 3-10). The population data for second-generation vermiform juveniles at 28 days after inoculation were not plotted in order to emphasize that the total numbers of first-generation nematodes in Black Valentine roots were similar in plants grown under all temperature regimes (Figure 3-6). The numbers of second-generation vermiform juveniles present in plants grown at 28/28°C were 261 (trial 1) and 253 (trial 2); the numbers in plants at 24/28°C were lower, at 101 and 17 in trials 1 and 2, respectively (data not shown). Males of *M. arenaria* race 1 were not observed in roots of Black Valentine or G1805 plants at any of the temperature regimes in either trial 1 or 2.

The effects of pre-inoculation conditioning temperatures on the numbers of *M. arenaria* race 1 penetrating roots and their subsequent development in roots of Black Valentine and G1805 plants were variable (Figures 3-7 and 3-8). Total numbers of nematodes and their rates of development in roots of Black Valentine plants conditioned at the pre-inoculation temperature of 24°C and 28°C were similar ($P < 0.05$). In trial 1, pre-inoculation temperature had no significant effect on the numbers of nematodes at each developmental stage in roots of Black Valentine plants (Figure 3-7). In the Black Valentine roots during trial 2, however, at 3 days after inoculation, there were 30% more nematodes present in the vermiform stage following pre-inoculation conditioning at 28°C than following pre-inoculation conditioning at 24°C ($P < 0.05$); similarly, at 14 days after inoculation, there were 58% more nematodes present in the adult stage following conditioning at the higher temperature than at the lower temperature ($P < 0.05$; Figure 3-8).

Similar to Black Valentine, the effect of pre-inoculation conditioning temperature on nematode penetration and development in G1805 roots also was variable and limited. In trial 1, at 1 and 2 days after inoculation, more nematodes penetrated the roots of plants pre-conditioned at 28°C than at 24°C ($P < 0.05$; Figure 3-7). Also at 28 days after inoculation, 50% more nematodes reached the adult stage (trial 1) and the swollen stage (trial 2) at 28°C compared with 24°C (Figures 3-7 and 3-8).

Unlike pre-inoculation conditioning temperatures for which effects were sporadic, post-inoculation incubation temperatures had a pronounced effect on the development of *M. arenaria* race 1 in the roots of both Black Valentine and G1805 plants. In both trials 1 and 2, the numbers of vermiform juveniles that penetrated the roots of Black Valentine plants 1, 2, and 3 days after inoculation were not significantly influenced by post-inoculation temperature ($P > 0.05$; Figures 3-9 and 3-10). However, in Black Valentine roots in trial 1, at 7 days after inoculation, there were

33% more nematodes in the swollen stage at 24°C than at 28°C; also, a larger number of nematodes (94%) had developed to the globose stage in the roots of plants incubated at 28°C than in plants incubated at 24°C. At 14 days after inoculation in trial 1, adult stages were present in roots of plants incubated at 28°C but not at 24°C ($P < 0.05$); in trial 2, they were present at similar levels in roots of plants incubated at both temperatures ($P < 0.05$). However, at 14 days after inoculation in both trials there were an average of 62% more swollen and 65% more globose stages remaining (did not develop into adults) in Black Valentine roots grown at 24°C than at 28°C ($P < 0.05$; Figures 3-9 and 3-10). By 21 days after inoculation, numbers of nematodes still present in the globose stage were 90% (trial 1) and 74% (trial 2) higher in plants incubated at 24°C than at 28°C ($P < 0.05$). By 28 days after inoculation, a second generation of vermiform juveniles had penetrated the roots of plants grown at 28°C in both trials. This second generation was absent from plants grown at 24°C in trial 1 (Figure 3-9); a small number (nine) were present at this temperature in trial 2 (Figure 3-10). However, the number of second-generation vermiform juveniles was significantly greater at 28°C than at 24°C in trial 1 ($P < 0.05$) and in trial 2 ($P < 0.10$).

Post-inoculation incubation temperatures also affected development of *M. arenaria* race 1 in roots of resistant G1805 plants. Similar to Black Valentine roots, numbers of vermiform juveniles penetrating G1805 roots 1, 2, and 3 days after inoculation were not significantly influenced by post-inoculation incubation temperature during either trial (Figures 3-9 and 3-10). Numbers of vermiform juveniles were greater in plants incubated at 24°C than at 28°C ($P < 0.05$) by 7 (45%) and 14 (90%) days after inoculation in trial 1 (Figure 3-9), and by 14 (50%) and 21 (60%) days after inoculation in trial 2 (Figure 3-10). By 7 days after inoculation in both the trials, an average of 92% more nematodes had developed to the swollen stage at 28°C than at 24°C ($P < 0.05$); development to globose stages was greater at 28°C than at 24°C by 14, 21, and 28 days after inoculation in trial 1 (average 75%), and by 14 and 21 days after inoculation in trial 2 (average 94%) ($P < 0.05$). Adult nematodes were present at higher levels at 28°C than at 24°C at 28 days after inoculation in trial 1, and at 14 days after inoculation in trial 2, although actual numbers of adults were still very low, only two (Figure 3-9) and one (Figure 3-10), respectively.

High Temperature Experiment 1

Growth of both Black Valentine and Nemasnap plants was affected by the high incubation temperatures of 30°C and 32°C. The mean fresh weights of Black Valentine plants, pooled over both harvest times, was greater at 30°C (7.63 g) than at 32°C (3.89 g) ($P < 0.05$); whereas, the mean plant weights of Nemasnap, pooled over both harvest times, were not different between 30°C (4.37 g) and 32°C (2.96 g) ($P > 0.05$; data not shown). At both harvest times and temperatures, the majority (>65%) of the *M. incognita* race 2 populations within the roots of Black Valentine and Nemasnap plants were present in the adult developmental stage (Table 3-2). At these times, only small numbers of nematodes were present in immature stages in both germplasms at both temperatures. However, at 22 days after inoculation at both 30°C and 32°C, the total populations of nematodes in the roots of Nemasnap plants were smaller than the total populations in the roots of Black Valentine plants ($P < 0.05$).

High Temperature Experiment 2

Growth of Black Valentine and G1805 plants was affected by the high incubation temperatures of 30°C and 32°C. The mean fresh weights of Black Valentine plants, pooled over both harvest times, was greater at 30°C (8.62 g) compared with that at 32°C (5.93 g) ($P < 0.05$); the mean plant weights of G1805, pooled over both harvest times, also were greater at 30°C (5.89 g) than at 32°C (4.18 g) ($P < 0.10$; data not shown). At both harvest times and temperatures, the majority (>64%) of the *M.*

arenaria race 1 population in roots of Black Valentine plants were present in the adult stage (Table 3-3). Development of the nematodes to adults in roots of G1805 plants did occur at both 30°C and 32°C; however, the actual numbers of nematodes present in these root systems were very small (Table 3-3). At 13 and 22 days after inoculation at both temperatures, the total populations of *M. arenaria* race 1 in roots of G1805 plants were smaller than those in the roots of Black Valentine plants ($P < 0.05$).

Discussion

At all four temperature regimes used in these experiments, resistance gene system 1 in Nemasnap was effective against *Meloidogyne incognita* race 2, and resistance gene system 2 in G1805 was effective against *M. arenaria* race 1. Resistance was demonstrated by the lower total numbers of nematodes in the roots of the resistant germplasms compared with the total numbers in the roots of the susceptible cultivar Black Valentine. These results confirm previous work by the author (chapter 2) and by Fassuliotis *et al.* (1970). Numbers of both *M. incognita* race 2 and *M. arenaria* race 1, in Nemasnap and G1805, respectively, reached maximum levels by 3 days after inoculation and declined thereafter. However, populations of these nematodes in roots of Black Valentine continued to increase for the duration of the experiments, indicating that nematodes entered the roots of the susceptible germplasm over an extended period of time. The decline in population levels in the roots of the resistant germplasms may be due to an inability to establish normal feeding sites (J. S. Huang, 1985).

A second generation of vermiform juveniles of *M. incognita* race 2 and *M. arenaria* race 1 developed in the roots of Black Valentine plants grown at the temperature regimes of 24/28°C and at 28/28°C. This second generation was greater in plants grown at 28/28°C than at 24/28°C in spite of the fact that the pre-inoculation conditioning of plants at both temperature regimes was for an equal number of accumulated heat units to ensure all plants were of a similar physiological age at the time of inoculation. The reason for the larger second generation in plants grown at 28/28°C than at 24/28°C is unclear, but possibly could be due to early temperature stress on the plant, leading to differences in nutrient availability and/or quality; however, this hypothesis was not tested.

Temperature and nutrient stress in plants have been reported to cause sex reversal in *Meloidogyne* spp., which results in a higher proportion of males in the population (Triantaphyllou, 1973). It also has been reported that fewer males of *Meloidogyne* spp. develop in plants grown at higher temperatures (Davide and Triantaphyllou, 1967). Males of *M. incognita* race 2 developed in the roots of Black Valentine and Nemasnap plants grown at all temperature regimes; however, pre- and post-inoculation temperatures of 24°C and 28°C had no consistent effect on the number of males in the roots of susceptible or resistant plants. Thus, it is not possible to determine if temperature stress on the plants reduced development of males or increased the process of sex reversal.

The effects of pre-inoculation conditioning temperatures on resistance expression in Nemasnap and G1805 plants to a later infection of *M. incognita* race 2 and *M. arenaria* race 1, respectively, were infrequent and variable. Although an effect of high and low early incubation temperatures and duration on resistance expression have been reported by Mullin *et al.* (1991a), their work was conducted on nematode-infected plants. There is no evidence from my studies that pre-inoculation temperature had a direct effect on resistance expression to a later infection by root-knot nematode. Thus, pre-inoculation temperature does not appear to modify the ability of a bean plant to resist root-knot nematodes at the temperatures tested.

Post-inoculation incubation temperature effects on nematode development were more pronounced than were pre-inoculation conditioning temperature effects. In all genotypes and in all tests, development of *M. incognita* race 2 and *M. arenaria* race 1 to the adult stage generally was more rapid at the higher post-inoculation temperature of 28°C compared with development at 24°C. Because this trend of more rapid development at 28°C occurred in both of the resistant germplasms and in the susceptible germplasm, it appears that the effect of post-inoculation incubation temperature is on nematode development rather than on resistance expression of either gene system 1 or gene system 2.

The results of my studies with gene system 2 resistance in G1805 demonstrated that resistance to *M. arenaria* race 1 was not reduced or lost at 28°C, at 30°C, or even at 32°C; the populations of nematodes were always very low and few individuals developed to adults. Omwega *et al.* (1990a) also reported stable resistance at 24°C to 30°C in G1805 to *M. incognita* race 1 and *M. javanica*, even though reproduction of *M. javanica* increased as temperature increased from 28°C to 30°C.

The results of this investigation also showed that gene system 1 resistance in Nemasnap to *M. incognita* race 2 was stable at 28°C, 30°C and 32°C; however, development to mature adults with egg masses increased as temperature increased from 24°C to 32°C. Omwega *et al.* (1990a) demonstrated that resistance to *M. incognita* race 2 in two of three germplasms containing gene system 1 (Alabama #1 and PI 165435) was lost at 30°C, and gradually reduced in all three germplasms (Alabama #1, PI 165426 and PI 165435) as temperature was increased to 30°C. Another resistant germplasm, A211, has been shown to be susceptible to *M. incognita* at 24°C when comparing egg mass and total egg production with those on a susceptible cultivar (Mullin *et al.*, 1991a). These differences between results may be explained by the effect of the plant genetic background on the effect of temperature on resistance expression (Canto-Sáenz, 1985). The more similar observations of temperature effects on resistance expression between PI 165426 and Nemasnap may be due to the fact that PI 165426 was used as the resistant parent in the development of the cultivar Nemasnap (Wyatt, 1983).

Regardless of the resistance mechanisms involved at temperatures of 28°C and higher, these are not the optimum temperatures for bean growth. In practice, most common beans are produced in regions where the temperatures range from 16°C to 24°C, within the range at which resistance has been documented to be stable (Fassuliotis *et al.*, 1970; Omwega *et al.*, 1990a). However, as temperatures in bean growing areas may reach higher than optimum levels during part of the growing season, germplasm with heat-stable resistance to root-knot nematodes may be important in *Meloidogyne* spp. management.

Table 3-1. Numbers of males of *Meloidogyne incognita* race 2 in root systems of susceptible Black Valentine and resistant Nemasnap common bean (gene system 1) incubated at the pre-inoculation conditioning temperatures and post-inoculation incubation temperatures of 24°C and 28°C.

		Numbers of males of *M. incognita* race 2			
	Harvest	Pre-inoculation temperature		Post-inoculation temperature	
Germplasm	day	24°C	28°C	24°C	28°C
Trial 1					
Black Val.	14	0	1	0	1
	21	2	1	1	2
	28	0	1	1	0*
Nemasnap	14	1	1	0	1
	21	4	8	5	6*
	28	5	4	5	3
Trial 2					
Black Val.	14	0	0	0	0
	21	1	1	1	1
	28	3	0*	1	2*
Nemasnap	14	2	1	0	3
	21	6	11	8	10
	28	5	10*	12	3*

Data are means of four replications.

Within each pre-/post-inoculation time, germplasm and harvest time, asterisks (*) indicate significantly different ($P < 0.05$) numbers of males in roots grown at 28°C compared with numbers of males in roots grown at 24°C.

Table 3-2. Numbers of *Meloidogyne incognita* race 2 in four developmental stages in root systems of susceptible Black Valentine and resistant Nemasnap common bean (gene system 1) incubated at temperatures of 30°C or 32°C.

Incubation temperature	Harvest day	Germplasm	Developmental stages of *M. incognita* race 2				
			Vermiform	Swollen	Globose	Adult	Total
30°C	13	Black Val.	0	4	20	118	142
		Nemasnap	0	2	9	31	42
	22	Black Val.	1	1	5	169	176
		Nemasnap	0	4	3	13	20*
32°C	13	Black Val.	0	3	6	67	76
		Nemasnap	0	1	4	14	19
	22	Black Val.	0	0	0	77	77
		Nemasnap	0	0	1	9	10*

Data are means of three replications.

Asterisks (*) indicate significantly different ($P < 0.05$) total numbers of nematodes in roots of Nemasnap compared with the total numbers in roots of Black Valentine at the same temperature and harvest time.

Table 3-3. Numbers of *Meloidogyne arenaria* race 1 in four developmental stages in root systems of susceptible Black Valentine and resistant G1805 common bean (gene system 2) incubated at temperatures of 30°C or 32°C.

Incubation temperature	Harvest day	Germplasm	Developmental stages of *M. arenaria* race 1: Vermiform	Swollen	Globose	Adult	Total
30°C	13	Black Val.	2	19	25	82	128
		G1805	0	10	1	1	12*
	22	Black Val.	1	3	19	95	118
		G1805	0	3	2	0	5*
32°C	13	Black Val.	0	8	22	43	73
		G1805	1	2	7	5	15*
	22	Black Val.	0	1	1	74	76
		G1805	0	2	2	6	10*

Data are means of three replications.

Asterisks (*) indicate significantly different ($P < 0.05$) total numbers of nematodes in roots of G1805 compared with the total numbers in roots of Black Valentine at the same temperature and harvest time.

Figure 3-1. Total populations of *Meloidogyne incognita* race 2 in susceptible Black Valentine and resistant Nemasnap common bean at four temperature regimes of pre-inoculation/post-inoculation temperatures of 24/24°C, 24/28°C, 28/24°C, and 28/28°C (gene system 1, trials 1 and 2).
Data are means of four replications at each harvest time.
Population data for the second generation of vermiform juveniles are not plotted for Black Valentine plants 28 days after inoculation in trials 1 and 2.

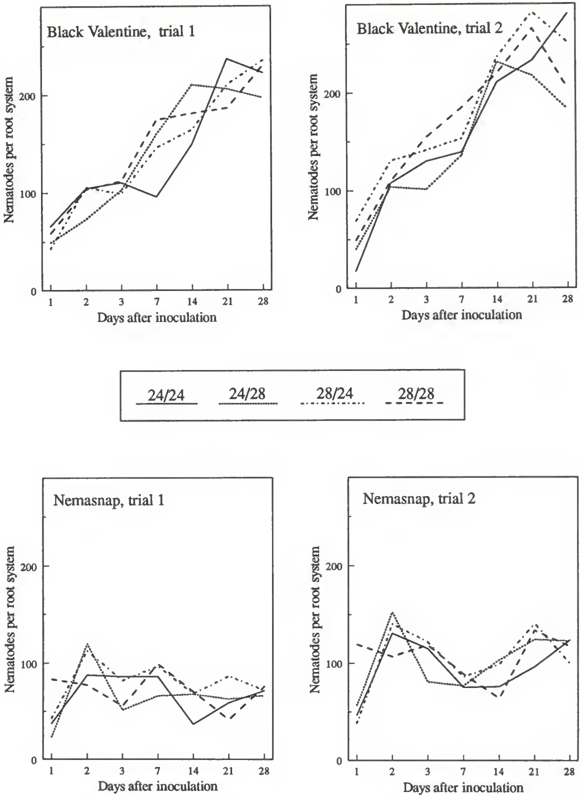

Black Valentine, trial 1
Black Valentine, trial 2
Nematodes per root system
0
100
200
1
2
3
7
14
21
28
Days after inoculation
24/24
24/28
28/24
28/28
Nemasnap, trial 1
Nemasnap, trial 2

Figure 3-2. Effects of pre-inoculation temperatures (24°C and 28°C) on penetration and development of *Meloidogyne incognita* race 2 in susceptible Black Valentine and resistant Nemasnap common bean germplasm (gene system 1, trial 1).
Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time.
At each harvest day within each germplasm, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants conditioned at the other pre-inoculation temperature.

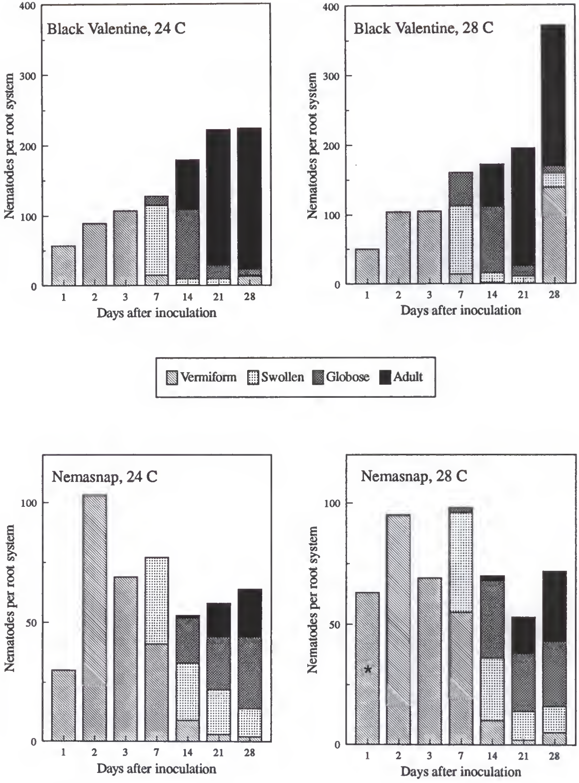

Black Valentine, 24 C
Black Valentine, 28 C
Nematodes per root system
0
100
200
300
400
1
2
3
7
14
21
28
Days after inoculation
Vermiform
Swollen
Globose
Adult
Nemasnap, 24 C
Nemasnap, 28 C
0
50
100
*

Figure 3-3. Effects of pre-inoculation temperatures (24°C and 28°C) on penetration and development of *Meloidogyne incognita* race 2 in susceptible Black Valentine and resistant Nemasnap common bean germplasm (gene system 1, trial 2).

Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time.

At each harvest day within each germplasm, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants conditioned at the other pre-inoculation temperature.

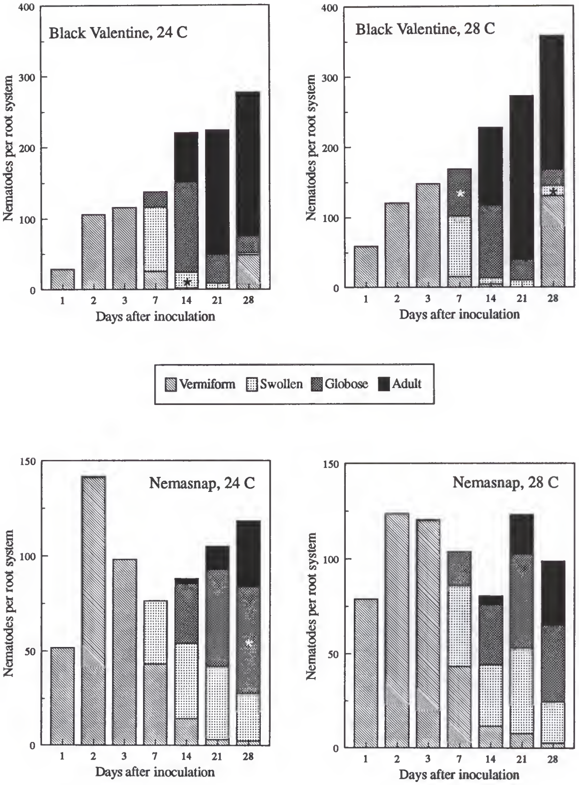

Black Valentine, 24 C
Black Valentine, 28 C
Nematodes per root system
0
100
200
300
400
1
2
3
7
14
21
28
Days after inoculation
Vermiform
Swollen
Globose
Adult
Nemasnap, 24 C
Nemasnap, 28 C
0
50
100
150
*

Figure 3-4. Effects of post-inoculation temperatures (24°C and 28°C) on penetration and development of *Meloidogyne incognita* race 2 in susceptible Black Valentine and resistant Nemasnap common bean germplasm (gene system 1, trial 1).

Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time.

At each harvest day within each germplasm, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants incubated at the other post-inoculation temperature.

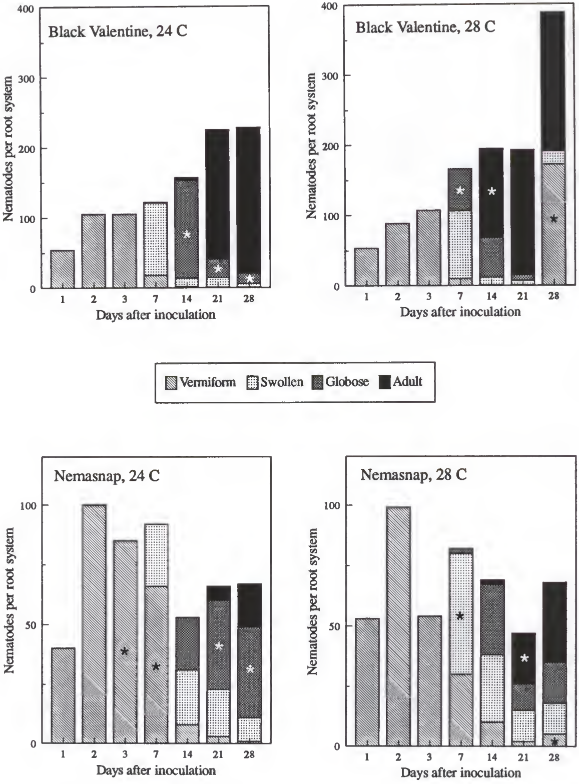

Black Valentine, 24 C
Black Valentine, 28 C
Nemasnap, 24 C
Nemasnap, 28 C
Nematodes per root system
Days after inoculation
0
50
100
200
300
400
1
2
3
7
14
21
28
*
▧ Vermiform
▦ Swollen
▩ Globose
■ Adult

Figure 3-5. Efects of post-inoculation temperatures (24°C and 28°C) on penetration and development of *Meloidogyne incognita* race 2 in susceptible Black Valentine and resistant Nemasnap common bean germplasm (gene system 1, trial 2).

Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time.

At each harvest day within each germplasm, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants incubated at the other post-inoculation temperature.

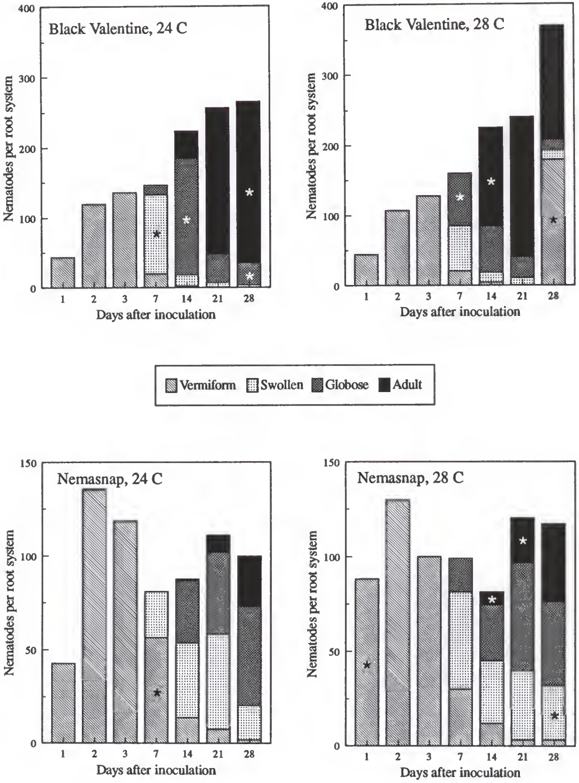

Black Valentine, 24 C
Black Valentine, 28 C
Nemasnap, 24 C
Nemasnap, 28 C
Nematodes per root system
Days after inoculation
0
100
200
300
400
50
150
1
2
3
7
14
21
28
Vermiform
Swollen
Globose
Adult
*

Figure 3-6. Total populations of *Meloidogyne arenaria* race 1 on susceptible Black Valentine and resistant G1805 common bean at four temperature regimes of pre-inoculation/post-inoculation temperatures of 24/24°C, 24/28°C, 28/24°C, and 28/28°C (gene system 2, trials 1 and 2).

Data are means of four replications at each harvest time.

Population data for the second generation of vermiform juveniles are not plotted for Black Valentine plants 28 days after inoculation in trials 1 and 2.

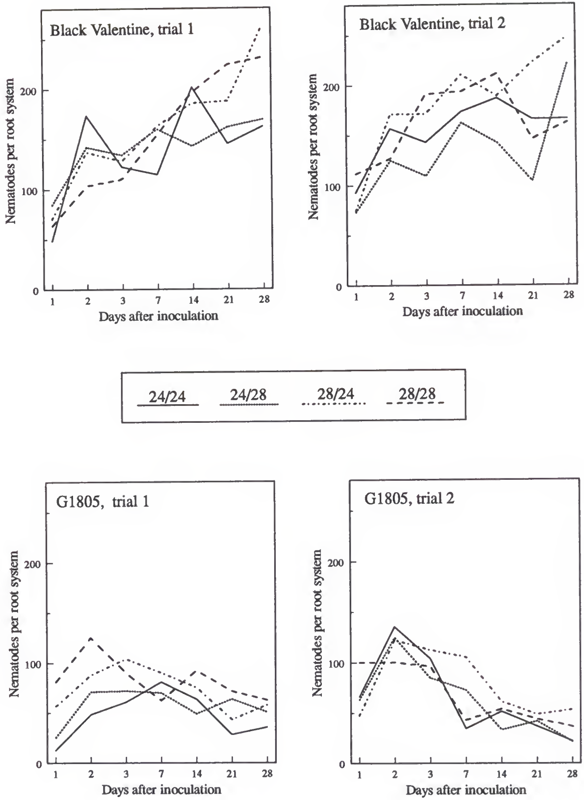

Black Valentine, trial 1
Black Valentine, trial 2
Nematodes per root system
0
100
200
1
2
3
7
14
21
28
Days after inoculation
24/24
24/28
28/24
28/28
G1805, trial 1
G1805, trial 2

Figure 3-7. Effects of pre-inoculation temperatures (24°C and 28°C) on penetration. and development of *Meloidogyne arenaria* race 1 in susceptible Black Valentine and resistant G1805 common bean germplasm (gene system 2, trial 1).

Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time.

At each harvest day within each germplasm, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants conditioned at the other pre-inoculation temperature.

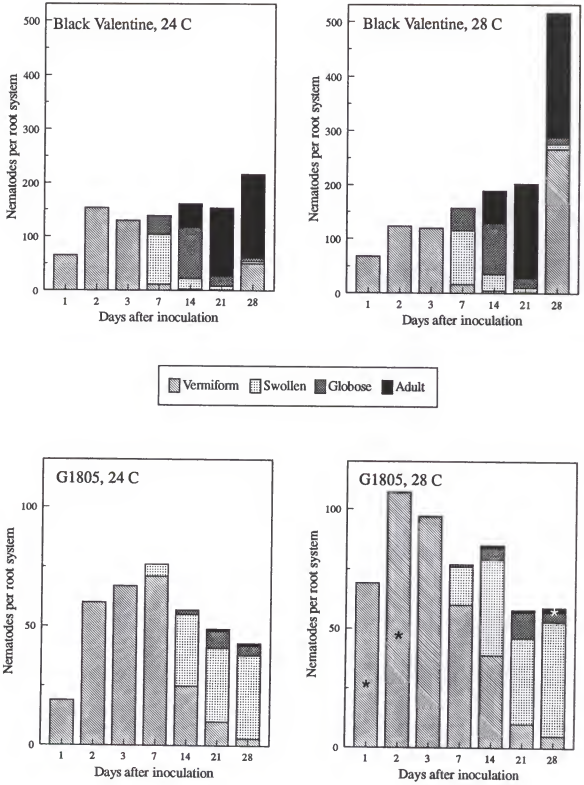

Black Valentine, 24 C
Black Valentine, 28 C
G1805, 24 C
G1805, 28 C
Nematodes per root system
Days after inoculation
0
100
200
300
400
500
50
1
2
3
7
14
21
28
*
▧ Vermiform
▦ Swollen
▩ Globose
■ Adult

Figure 3-8. Effects of pre-inoculation temperatures (24°C and 28°C) on penetration. and development of *Meloidogyne arenaria* race 1 in susceptible Black Valentine and resistant G1805 common bean germplasm (gene system 2, trial 2).

Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time.

At each harvest day within each germplasm, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants conditioned at the other pre-inoculation temperature.

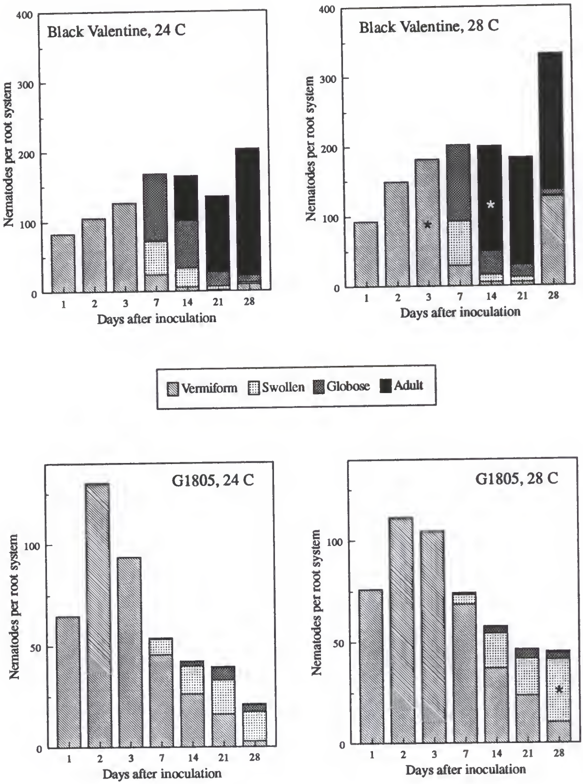

Black Valentine, 24 C
Black Valentine, 28 C
G1805, 24 C
G1805, 28 C
Nematodes per root system
Days after inoculation
0
50
100
200
300
400
1
2
3
7
14
21
28
*
▨ Vermiform
▦ Swollen
▩ Globose
■ Adult

Figure 3-9. Effects of post-inoculation temperatures (24°C and 28°C) on penetration and development of *Meloidogyne arenaria* race 1 in susceptible Black Valentine and resistant G1805 common bean germplasm (gene system 2, trial 1).
Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time.
At each harvest day within each germplasm, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants incubated at the other post-inoculation temperature.

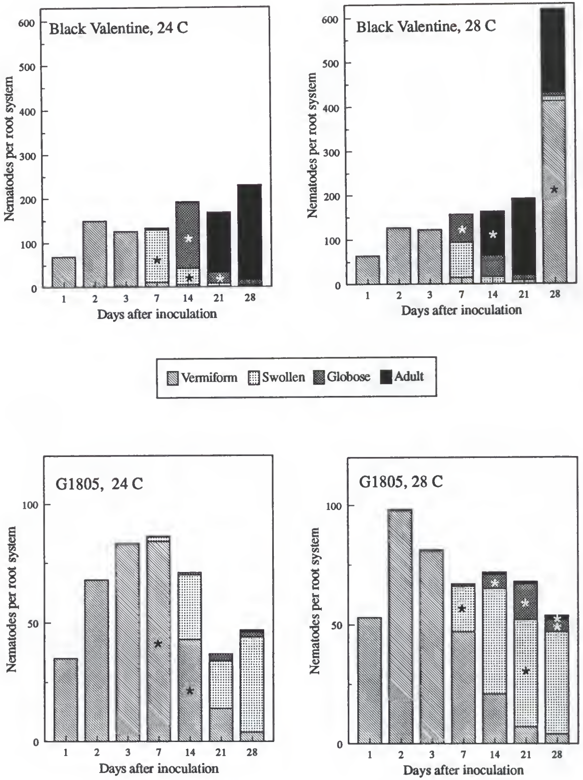
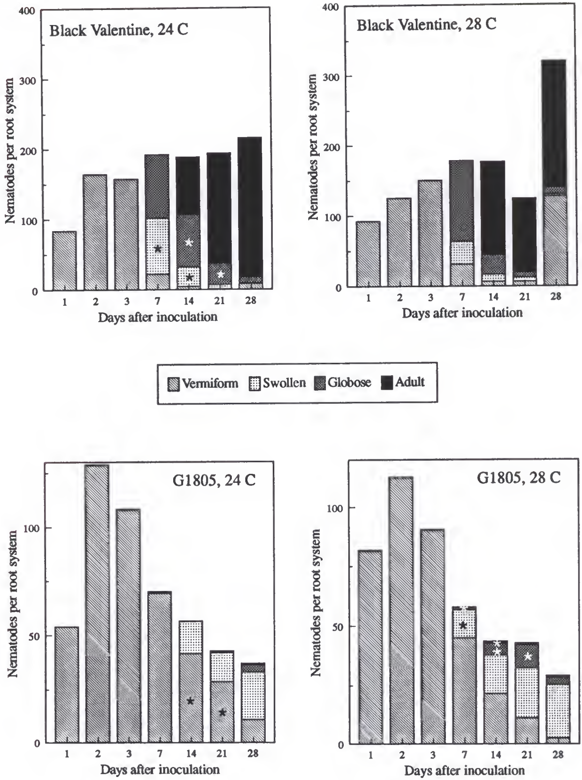

Black Valentine, 24 C
Nematodes per root system
0
100
200
300
400
500
600
1
2
3
7
14
21
28
Days after inoculation
Black Valentine, 28 C
Nematodes per root system
0
100
200
300
400
500
600
1
2
3
7
14
21
28
Days after inoculation
Vermiform
Swollen
Globose
Adult
G1805, 24 C
Nematodes per root system
0
50
100
1
2
3
7
14
21
28
Days after inoculation
G1805, 28 C
Nematodes per root system
0
50
100
1
2
3
7
14
21
28
Days after inoculation

Figure 3-10. Efects of post-inoculation temperatures (24°C and 28°C) on penetration and development of *Meloidogyne arenaria* race 1 in susceptible Black Valentine and resistant G1805 common bean germplasm (gene system 2, trial 2).

Data are means of eight replications, pooled from the respective temperature regimes, at each harvest time.

At each harvest day within each germplasm, asterisks (*) indicate significantly higher ($P < 0.05$) numbers of nematodes in a developmental stage compared with numbers in the same developmental stage in plants incubated at the other post-inoculation temperature.


Black Valentine, 24 C
Black Valentine, 28 C
G1805, 24 C
G1805, 28 C
Nematodes per root system
Days after inoculation
0
100
200
300
400
50
1
2
3
7
14
21
28
▨ Vermiform
▦ Swollen
▩ Globose
■ Adult
*

CHAPTER 4
RESEARCH SUMMARY AND CONCLUSIONS

Resistance to *Meloidogyne* spp. in *Phaseolus vulgaris* germplasm, controlled by either gene system 1 or gene system 2, was expressed as a post-infection mechanism. Resistance was effective both in reducing the total populations of root-knot nematodes within the roots of resistant plants and in delaying nematode development compared with a susceptible germplasm. Pre-infection resistance mechanisms did not appear to be effective, since numbers of nematodes penetrating roots of resistant and susceptible plants were similar in all of the studies conducted. The resistance responses of gene system 1 and gene system 2 appeared to be similar in their post-infection mechanism of delaying nematode development. However, gene system 2 resistance, controlled by a single dominant gene, was more restrictive than gene system 1 resistance, which is controlled by two or more interacting recessive genes.

Efficacy of gene system 1 resistance in snap beans has been demonstrated in field trials (Wyatt *et al.,* 1980) These workers showed that resistance was equivalent to the action of the soil fumigation treatment with 1,2-dibromo-3-chloropropane (DBCP) in reducing root galls and numbers of *M. incognita* nematodes in the soil. Resistance in common bean has importance as an economical and environmentally safe management tool for root-knot nematodes. Gene system 1 specifically conditions resistance to *M. incognita* races 2, 3, and 4, whereas gene system 2 conditions resistance to *M. arenaria, M. incognita* race 1, and *M. javanica*. Due to this specificity and the presence of mixed populations of *Meloidogyne* spp. in some agricultural soils, incorporation of the two gene systems into a single germplasm to provide resistance against all *Meloidogyne* species and races would greatly improve the management potential of resistance against root-knot nematodes in bean production areas throughout the world.

Resistance to *Meloidogyne* spp. in *P. vulgaris* was effective at all temperatures studied, with smaller populations of nematodes in roots of resistant plants compared with susceptible plants. Pre-inoculation temperature did not modify resistance expression of gene system 1 or gene system 2 to later infection by root-knot nematode. Post-inoculation temperature, however, did affect resistance expression; as temperature increased from 24°C to 28°C, development of the nematodes to adult stages was more rapid in the susceptible and both of the resistant germplasms. The effect of post-inoculation temperature appeared to be directly on nematode development rather than on resistance expression of either gene system 1 or gene system 2. Resistance expression also was stable at 30°C and 32°C.

The majority of common bean production occurs in regions where temperatures range from 16°C to 24°C; however, temperatures higher than the optimum range can occur during part of the growing season in many of these regions. Information on effects of temperature on the relationship between developmental behavior of *Meloidogyne* spp. and resistance in bean plants is important in order to predict how resistant germplasm would react under high soil temperatures. Such information would greatly improve the management potential of resistance against *Meloidogyne* spp. in bean-growing regions of the world.

The importance of investigating alternative methods of root-knot nematode control are especially important due to the ever increasing restrictions on the use of chemical control methods. Information about the resistance mechanisms against *Meloidogyne* spp. in common bean are important for the development of long-lasting and effective resistant germplasms for root-knot nematode management.

APPENDIX A
RESISTANCE IN GERMPLASMS OF *PHASEOLUS VULGARIS* TO FLORIDA POPULATIONS OF *MELOIDOGYNE* SPECIES

Introduction

The term resistance describes the effect of host genes on nematode reproduction (Cook and Evans, 1987). Virulence is the term used to describe the ability of a host race or pathotype to reproduce on a host resistant to other host races or pathotypes of that nematode species. Host races are distinguished by their ability or inability to reproduce on designated plant species; pathotypes of a nematode species or host race are distinguished by their ability to reproduce on designated genotypes of a host plant.

Resistance in *Phaseolus vulgaris* to root-knot nematodes has been demonstrated to be controlled by one of two genetic systems (Omwega *et al.*, 1989) (Table A-1). Gene system 1, found in the cultivars Alabama #1 and Nemasnap, and in the genotype PI 165426, conditions resistance to *M. incognita* (Kofoid and White) Chitwood races 2, 3, and 4; it is controlled by the interaction of two or more independent recessive genes (Blazey *et al.*, 1964; Fassuliotis *et al.,* 1970). Gene system 2, found in the Mexican accessions G2618 and G1805, and lines A315 and A445 bred from them, confers resistance to *M. incognita* race 1, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood races 1 and 2. Resistance in gene system 2 is controlled by a single dominant gene (Omwega *et al.*, 1990b).

Based on the specificity of the resistance gene systems in *P. vulgaris* germplasm, experiments were conducted to determine the resistant nature of several common bean germplasms (Table A-2) and to determine the virulent nature of several species and races of Florida isolates of root-knot nematodes (Table A-3).

Materials and Methods

Several experiments were conducted in a growth rooms and growth chambers maintained with a 10-hr daylength, and a mean day temperature of 25° C. The mean night temperatures were 22° C in the growth room, and 24° C in the growth chambers. In experiments 1, 2, 5, and 6, all root systems were stained with acid fuchsin (Byrd *et al.,* 1983). Total numbers of nematodes in the stained root systems were recorded at each harvest date, and individuals were assigned to one of four developmental stages based on readily visible characteristics. The first developmental stage (vermiform) included vermiform, non-swollen, second-stage juveniles; the second developmental stage (swollen) included swollen, sausage-shaped, second-stage juveniles; the third developmental stage (globose) included swollen, partially globose individuals with conical tails; the final developmental stage (adult) included fully globose pre-adults and adults with or without egg masses. In experiments 3 and 4, all root systems were rated visually for galls using a gall rating index of 0 - 5, such that 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = more than 100 galls per root system (Taylor and Sasser, 1978).

Gene System 1

Experiment 1

This experiment was conducted in a growth room to establish the resistant nature of two germplasms containing gene system 1 to a Florida population of *M. incognita* race 3. The three treatments were susceptible Black Valentine, resistant Alabama #1, and resistant Nemasnap inoculated with *M. incognita* race 3. Plants were grown two per pot and inoculated with 500 eggs per pot as described in the Materials and Methods section of Chapter 2. Each treatment was replicated five times. Two replications were harvested at 35 days after inoculation; the remaining replications were discarded.

Experiment 2

This experiment was conducted in a growth chamber to compare the virulent nature of the Florida population of *M. incognita* race 3 with that of a California population of *M. incognita* race 3 on resistant Nemasnap germplasm. The four treatments were susceptible Black Valentine and resistant Nemasnap germplasms inoculated with either a Florida or a California population of *M. incognita* race 3. Plants were grown in seedling growth pouches and inoculated with 1,500 second-stage juveniles of *M. incognita* race 3 as described in the Materials and Methods section of Chapter 3. Three replications of each treatment were harvested at 7 and 21 days after inoculation.

Experiment 3

This experiment was conducted in a growth room to establish resistance in germplasms containing gene system 1 to a Florida population of *M. incognita* race 2. The eight treatments were susceptible Black Valentine and seven resistant *P. vulgaris* germplasms containing gene system 1 (Table A-2). Plants were grown singly in pots and were inoculated with 900 second-stage juveniles of *M. incognita* race 2. Each treatment was replicated four times and plants were harvested at 28 days after inoculation.

Gene System 2

Experiment 4

This experiment was conducted simultaneously with experiment 3 to establish resistance in germplasms containing gene system 2 to a Florida population of *M. javanica*. The five treatments were susceptible Black Valentine and four resistant *P. vulgaris* germplasms containing gene system 2 (Table A-2). All plants were inoculated with 900 second-stage juveniles of *M. javanica.*

Experiment 5

This experiment was conducted in a growth room to determine the virulence of a Florida population of *M. arenaria* race 1 to a germplasm containing gene system 2. The treatments were susceptible Black Valentine and resistant G1805 germplasms inoculated with 1,000 second-stage juveniles of *M. arenaria* race 1. Each treatment was replicated 10 times. At 15 and 25 days after inoculation, a single replication of each treatment was harvested, and at 35 days after inoculation, five replications of each treatment were harvested.

Experiment 6

This experiment was conducted in a growth chamber to determine the virulence of a Florida population of *M. arenaria* race 2 to a germplasm containing gene system 2. The two treatments were susceptible Black Valentine and resistant G1805 germplasms inoculated with *M. arenaria* race 2. Plants were grown in seedling growth pouches and inoculated with 1,500 second-stage juveniles of *M. arenaria* race 2 as described previously. Four replications of each treatment were harvested at 7 and 21 days after inoculation.

Results

Gene System 1

Experiment 1

At 35 days after inoculation, no differences were observed among the total numbers of *M. incognita* race 3 or the numbers of nematodes at each developmental stage among the three germplasms ($P > 0.05$; Table A-4). Low numbers of adults, a mean of two, were observed in the roots of the susceptible and the resistant germplasms. Also at this time, the majority of nematodes, a mean of 56%, were present in the globose stage in all of the germplasms. Since the effect of resistance on nematode development was not observed at this time, the experiment was not continued.

Experiment 2

At 7 days after inoculation in the roots of Black Valentine plants, no differences were observed in total numbers or numbers at each developmental stage of the Florida and the California populations of *M. incognita* race 3 ($P > 0.05$; Table A-5). Also at this time in the roots of Nemasnap plants, no differences in total numbers or in numbers at the different developmental stages were observed between the two populations of *M. incognita* race 3 ($P > 0.05$). However, when the numbers at each developmental stage as a percentage of the total number in the roots are compared, a larger proportion of the Florida population (92%) than the California population (35%) were present in the swollen stage ($P < 0.05$).

At 21 days after inoculation in Black Valentine plants, again no differences in the total numbers of nematodes or the numbers at each developmental stage were observed between the two populations of *M. incognita* race 3 ($P > 0.05$). In roots of Nemasnap plants at this time, the total population of 385 nematodes of the Florida population was approximately four-fold greater than the total population of 102 nematodes of the California population ($P < 0.05$). Also at this time, 93% of the Florida population of *M. incognita* race 3 was present as adults in the roots of Nemasnap plants compared with only 10% of the California population present as adults ($P < 0.05$). A mean of 65% of the California population of *M. incognita* race 3 was still present as vermiform and swollen stages in Nemasnap roots at 21 days after inoculation.

Experiment 3

At 28 days after inoculation Black Valentine exhibited a susceptible gall index of 5.00 (Table A-6). The germplasms A211, Alabama #1, Nemasnap, PI 165426 (black and brown seed coats) and PI 165435 all exhibited gall indices of 2.00 or lower. The germplasm PI 165426 grown from seed with white coats demonstrated an intermediate gall index of 3.75.

Gene System 2

Experiment 4

At 28 days after inoculation, no resistance was observed in any of the four germplasms, A315, A445, G1805, and G2618 (Table A-7). The mean gall index rates ranged from 3.75 to 5.00 in the resistant germplasms; these were not different from the gall index of 5.00 for susceptible Black Valentine ($P < 0.05$).

Experiment 5

At 15 and 25 days after inoculation, the total numbers of *M. arenaria* and the numbers at each developmental stage indicated a trend of delayed development and resistance in G1805 compared with Black Valentine (Table A-8). At 35 days after inoculation, a significantly larger population of 435 nematodes was present in the roots of Black Valentine plants compared with a population of only 53 nematodes in roots of

G1805 plants ($P < 0.05$). The delay in development to mature stages was demonstrated by the majority, 77%, of nematodes present in the vermiform and swollen stages in roots of G1805 plants compared with less than 1% present in these stages in roots of Black Valentine plants ($P < 0.05$). A total of only seven nematodes (13% of the total number) were present as adults in roots of resistant plants compared with 421 adults (97% of the total number) present in roots of susceptible plants ($P < 0.05$).

Experiment 6

At 7 days after inoculation, the total numbers of *M. arenaria* race 2 in the roots of Black Valentine and G1805 plants were not different (Table A-9). At this time however, the number of nematodes that had developed to the swollen stage was greater in Black Valentine plants (123 nematodes) than in G1805 plants (one nematode) ($P < 0.05$). At 21 days after inoculation, 91% of nematodes were present in the adult stage in roots of Black Valentine plants, whereas 94 % of nematodes were still present in the vermiform stage in roots of G1805 plants.

Discussion

In these studies, resistance in *Phaseolus vulgaris* germplasms to Florida populations of *Meloidogyne* species and races was variable. Germplasms containing resistance gene system 1 demonstrated similar resistant behavior to a Florida population of *M. incognita* race 2 as previously reported by Fassuliotis *et al.* (1970) and by Omwega *et al.* (1989) (Table A-1). Germplasms containing resistance gene system 2 were resistant to Florida populations of both *M. arenaria* race 1 and race 2, also as reported by Omwega *et al.* (1989) (Table A-1). However, the virulent behavior of Florida populations of *M. incognita* race 3 and *M. javanica* in germplasms containing resistance gene system 1 and gene system 2, respectively, was different from that previously reported (Omwega *et al.*, 1989).

Results of a comparison study between the Florida population of *M. incognita* race 3 and a California population of *M. incognita* race 3, against which gene system 1 resistance previously was demonstrated to be effective, indicate that the Florida population is a virulent pathotype able to reproduce on this resistant germplasm. The Florida population of *M. javanica* may also be a virulent pathotype on bean germplasm containing resistance gene system 2; however, this was not tested in comparison studies with other populations of *M. javanica.*

Germplasm PI 165426 grown from seed with white seed coats expressed susceptibility to *M. incognita* race 2; however, the same germplasm grown from seed with black or brown seed coats expressed resistance to the same population of the nematode. It appears as though resistance expression in this case is associated with pigmentation in the seed coat rather than a pathotype of *M. incognita* race 2 being virulent to this particular line of PI 165426.

With the increasing demand for management methods not dependent on chemicals, development of resistant germplasm for use in crop production is of growing importance. However, as these studies demonstrate, it is critical to assess the performance of resistant germplasm to a wide range of geographically localized populations of nematode species and races in order to identify potentially virulent pathotypes.

Table A-1. Resistance in *Phaseolus vulgaris* germplasm to *Meloidogyne* species and races (adapted from Omwega *et al.*, 1989).

P. vulgaris	*M. arenaria*		*M. hapla*	*M. incognita*				*M. javanica*
germplasm	1	2		1	2	3	4	
Gene System 1								
Alabama #1	S	S	R	S	R	R	R	S
Nemasnap	S	-	-	-	R	R	-	-
PI 165426	S	S	R	S	R	R	R	S
Gene System 2								
G1805	R	R	-	R	S	-	-	R
G2618	R	R	S	R	-	-	-	R
A315	R	R	S	R	S	S	S	R
A445	R	R	R	R	S	S	S	R

R = resistant, S = susceptible, - = not tested.

Table A-2. Origins and sources of *Phaseolus vulgaris* germplasm.

Germplasm	Origin	Source
Susceptible		
Black Valentine	USA	Phipps Ranch, Pascadero, CA
Resistance Gene System 1		
A211	Colombia	CIAT, Cali, Colombia
Alabama #1	USA	Hastings Seed Co., Atlanta, GA
Nemasnap	USA	US Vegetable Laboratory, Charleston, SC
PI 165426 (black seed)	Mexico	CIAT, Cali, Colombia
PI 1655426 (brown seed)	Mexico	Western Regional Plant Introduction Center, Pullman, WA
PI 165426 (white seed)	Mexico	CIAT, Cali, Colombia
PI 165435	Mexico	Western Regional Plant Introduction Center, Pullman, WA
Resistance Gene System 2		
A315	G2618	CIAT, Cali, Colombia
A445	G1805	CIAT, Cali, Colombia
G1805	Mexico	CIAT, Cali, Colombia, University of California, Riverside, CA
G2618	Mexico	CIAT, Cali, Colombia

Table A-3. Sources of *Meloidogyne* species.

Meloidogyne spp.	Source
M. arenaria race 1	Peanut field, Williston, FL
M. arenaria race 2	The Land Pavilion, EPCOT, Orlando, FL
M. incognita race 2	Tobacco field, Alachua Co., FL
M. incognita race 3	Field, FL
M. incognita race 3	University of California, Riverside, CA
M. javanica	Tobacco field, Alachua Co., FL

Table A-4. Resistance in *Phaseolus vulgaris* germplasm containing gene system 1 to a Florida population of *Meloidogyne incognita* race 3.

P. vulgaris	Developmental stages of *M. incognita* race 3				Total
germplasm	Vermiform	Swollen	Globose	Adult	numbers
Black Valentine	2	16	28	3	48
Alabama #1	4	17	20	1	40
Nemasnap	2	16	28	2	48

Data are means of two replications, two plants per pot.

No significant differences ($P < 0.05$) were observed among the three germplasms.

Table A-5. Resistance in *Phaseolus vulgaris* germplasm containing gene system 1 to a Florida and a California population of *Meloidogyne incognita* race 3.

P. vulgaris germplasm	*M. incognita* race 3 source	Developmental stages of *M. incognita* race 3 Vermiform	Swollen	Globose	Adult	Total numbers
7 days after inoculation						
Black Valentine	Florida	78	237	10	0	325
	California	20	188	18	0	226
Nemasnap	Florida	11	125	0	0	136
	California	82	44	0	0	126
21 days after inoculation						
Black Valentine	Florida	2	2	5	660	669
	California	8	1	28	742	779
Nemasnap	Florida	2*	5*	20	358**	385**
	California	37	29	25	11	102

Data are means of three replications.

Asterisks (*, **) indicate significant differences ($P < 0.10$, $P < 0.05$) in numbers of a Florida population of *M. incognita* race 3 and numbers of a California population of *M. incognita* race 3, for various developmental stage on Nemasnap.

Table A-6. Resistance in *Phaseolus vulgaris* germplasm containing gene system 1 to a Florida population of *Meloidogyne incognita* race 2.

P. vulgaris germplasm	Mean root gall index
Black Valentine	5.00^{a}
A211	1.75^{c}
Alabama #1	2.00^{c}
Nemasnap	0.75^{c}
PI 165426 (black seed coat)	1.50^{c}
PI 165426 (brown seed coat)	1.25^{c}
PI 165426 (white seed coat)	3.75^{b}
PI 165435	1.75^{c}

Data are means of four replications. Values followed by the same letter are not different ($P < 0.05$) according to Duncan's multiple range test.

Table A-7. Resistance in *Phaseolus vulgaris* germplasm containing gene system 2 to a Florida population of *Meloidogyne javanica*.

P. vulgaris germplasm	Mean root gall index
Black Valentine	5.00[a]
A315	4.75[a]
A445	3.25[a]
G1805	4.25[a]
G2618	5.00[a]

Data are means of four replications. Values followed by the same letter are not different ($P < 0.05$) according to Duncan's multiple range test.

Table A-8. Resistance in *Phaseolus vulgaris* germplasm containing gene system 2 to a Florida population of *Meloidogyne arenaria* race 1.

P. vulgaris germplasm	Developmental stages of *M. arenaria* race 1				Total numbers
	Vermiform	Swollen	Globose	Adult	
15 days after inoculation[†]					
Black Valentine	2	75	38	0	115
G1805	7	8	1	0	16
25 days after inoculation[†]					
Black Valentine	1	19	27	136	183
G1805	18	41	21	2	82
35 days after inoculation[‡]					
Black Valentine	0	2	12	421	435
G1805	5*	36*	5*	7*	53*

[†] Data are from one replication.

[‡] Data are means of five replications.

At 35 days after inoculation, asterisks (*) indicate significantly different ($P < 0.05$) numbers of nematodes in each developmental stage in roots of G1805 plants compared with numbers in roots of Black Valentine plants.

Table A-9. Resistance in *Phaseolus vulgaris* germplasm containing gene system 2 to a Florida population of *Meloidogyne arenaria* race 2.

P. vulgaris	Developmental stages of *M. arenaria* race 2				Total
germplasm	Vermiform	Swollen	Globose	Adult	numbers
7 days after inoculation					
Black Valentine	117	123	0	0	240
G1805	136	1*	0	0	137
21 days after inoculation					
Black Valentine	3	6	17	257	283
G1805	58*	4	0	0*	62*

Data are means of four replications.

Asterisks (*) indicate significantly different ($P < 0.05$) numbers of nematodes in each developmental stage in roots of G1805 plants compared with numbers in roots of Black Valentine plants.

APPENDIX B
HISTOPATHOLOGY OF *MELOIDOGYNE ARENARIA* RACE 1 INFECTION IN RESISTANT AND SUSCEPTIBLE *PHASEOLUS VULGARIS* GERMPLASM

Introduction

Giant cells are specialized feeding cells induced and maintained in roots of susceptible plants by the feeding of root-knot nematodes (Hussey, 1985). The formation and development of characteristic multinucleate giant cells in roots of susceptible plants infected with *Meloidogyne* spp. have been recorded for many plants, among them *Impatiens* sp. (Jones and Payne, 1978), soybean (Dropkin and Nelson, 1960), and tomato (Bird, 1962). Parenchyma cells at the head of the sedentary juvenile in the undifferentiated vascular tissue are induced to undergo mitosis without subsequent cytokinesis, which results in giant cell formation (C. S. Huang, 1985; Huang and Maggenti, 1969, Jones and Payne, 1978).

Normal patterns of giant cell induction and development have been reported absent from roots of resistant plants infected with *Meloidogyne* spp. (Dropkin and Nelson, 1960; Dropkin *et al.*, 1969; Fassuliotis *et al.*, 1970; McClure *et al.*, 1974). A hypersensitive reaction is a common response of resistant plants to infection by root-knot nematodes. Cells around the head of the sedentary juvenile not only fail to develop, but they become necrotic and die, thus limiting the area of root injury and preventing further nematode development. This necrotic hypersensitive reaction has been observed in soybean (Dropkin and Nelson, 1960) and in tomato (Dropkin *et al.*, 1969). A second response to *Meloidogyne* spp. infection observed in resistant plants is the early initiation of giant cells, but with limited cell enlargement and mitotic divisions. This response has been reported in common bean (Fassuliotis *et al.*, 1970), cotton

(McClure *et al.*, 1974), and soybean (Dropkin and Nelson, 1960). These abnormal giant cells were associated with poor nematode development, and often the cells disintegrate or became necrotic, thus preventing further nematode development (Dropkin and Nelson, 1960; Fassuliotis *et al.*, 1970; McClure *et al.*, 1974).

The correlation between giant cell health and development of *M. incognita* has been reported in both resistant and susceptible *P. vulgaris* germplasm (Fassuliotis *et al.*, 1970). However, this study was limited to a resistant germplasm containing gene system 1, which conditions resistance to *M. incognita* races 2, 3, and 4. Since this study, a second gene system (gene system 2) has been identified in common bean, which conditions resistance to *M. arenaria, M. incognita* race 1, and *M. javanica* (Omwega *et al.* 1989, 1990b). The purpose of the present investigation was to follow the histopathology of resistant bean roots (gene system 2) infected with *M. arenaria* race 1, in order to determine if this resistance was expressed as a hypersensitive reaction or by giant cell initiation followed by disintegration.

Materials and Methods

The experiment conducted in this study used the susceptible *P. vulgaris* cultivar 'Black Valentine' and the resistant germplasm G1805 (gene system 2), together with *M. arenaria* race 1. The origin of the bean germplasms and population of *M. arenaria* race 1 were as described previously (Materials and Methods, Chapter 2).

Plants were grown in seedling growth pouches and inoculated with 750 second-stage juveniles of *M. arenaria* race 1 as described previously (Materials and Methods, Chapter 3). All plants were grown in a growth chamber at 24°C, and four root systems of each germplasm were harvested at 2, 4, 6, 8, 10, and 15 days after inoculation. Root tips (2 - 6 days after inoculation) and selected root tips and segments (8 - 15 days after inoculation), all approximately 1 cm-long, were fixed in FAA, dehydrated and embedded in paraffin (Tables B-1 and B-2). Roots were sectioned at 10 µm, stained with saffranin and fast green, and mounted as described by Daykin and Hussey (1985).

Results

Longitudinal sections of root pieces of susceptible Balck Valentine, harvested 8 days after inoculation, were obtained and mounted on slides. However, sections of root pieces of resistant G1805, harvested 8 days after inoculation, were not obtained by longitudinal sectioning. The tissue within the wax collapsed during the sectioning procedure with the rotary microtome and no undamaged sections were obtained for mounting, staining or observation. Due to time constraints, the experiment was not continued.

Table B-1. Ethanol (EtOH) and tertiary butyl alcohol (TBA) dehydration series.

			Quantity (ml) needed for solution		
Step	Solution % alcohol	Time	100% EtOH	100% TBA	Water
1	50% EtOH/TBA	2 hrs	40	10	50
2	70% EtOH/TBA	overnight	50	20	30
3	85% EtOH/TBA	1-2 hrs	50	35	15
4	95% EtOH/TBA	1-2 hrs	45	55	0
5	100% EtOH/TBA	1-3 hrs	25	75	0
6	100% TBA	1-3 hrs	0	100	0
7	100% TBA	1-3 hr	0	100	0
8	100% TBA	overnight	0	100	0

Table B-2. Mineral oil, wax and paraffin infiltration series.

			Quantity (v:v) needed for solution		
Step	Solution	Time	100% TBA	Mineral oil	Paraffin wax
1	TBA : mineral oil	2 hrs	50	50	0
2	Wax : mineral oil	2 hrs	0	50	50
3	Wax : mineral oil	2 hrs	0	25	75
4	Wax	1-2 hrs	0	0	100
5	Wax	overnight	0	0	100
6	Wax	1-2 hrs	0	0	100
7	Wax (cool)	indefinite	0	0	100

TBA = tertiary butyl alcohol.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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